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METHODOLOGIC GUIDELINES FOR REPORTS OF CLINICAL TRIALS*

1. Authors should discuss briefly the quality control methods used to ensure that the data are complete and accurate. A reliable procedure should be cited for ensuring that all patients entered on study are actually reported upon. If no such procedures are in place, their absence should be noted. Any procedures employed to ensure that assessment of major endpoints is reliable should be mentioned (eg, second-party review of responses) or their absence noted.

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9. The methods of statistical analysis should be described in detail sufficient that a knowledgeable reader could reproduce the analysis if the data were available.

***From* Methodologic Guidelines for Reports of Clinical Trials (Cancer Treat Rep 69:1–3, 1985).

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Cancer Treatment Reports
Volume 70, Number 7
July 1986

Contents

ORIGINAL ARTICLES

827 Doxorubicin, Ifosfamide, and Dacarbazine (AID) With Mesna Uroprotection for Advanced Untreated Sarcoma: A Phase I Study. **A. D. Elias and K. H. Antman.** Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts

835 Pharmacokinetics of Aclarubicin and Its Metabolites in Humans and Their Disposition in Blood Cells. **S. Ando, T. Nakamura, D. Kagawa, T. Ueda, T. Nishimura, A. Kubo, H. Tsutani, M. Sasada, and H. Uchino.** Kyoto University, Japan

843 Phase II Trials of α-Difluoromethylornithine, an Inhibitor of Polyamine Synthesis, in Advanced Small Cell Lung Cancer and Colon Cancer. **M. D. Abeloff, S. T. Rosen, G. D. Luk, S. B. Baylin, M. Zeltzman, and A. Sjoerdsma.** The Johns Hopkins University School of Medicine, Baltimore, Maryland

847 Phase I Study of Continuous-Infusion Cisplatin. **M. R. Posner, A. T. Skarin, J. Clark, and T. J. Ervin.** Roger Williams General Hospital, Brown University, Providence, Rhode Island

851 Intratumoral Injection of Thymidine: Enhancement of the Antitumor Activity and Incorporation of 5-Fluorouracil Into Tumor RNA in a Murine Tumor System. **H. Matsuoka, H. Masuda, Y. Maehara, K. Sugimachi, and K. Inokuchi.** Kyushu University, Fukuoka, Japan

859 Human Plasma Pharmacokinetics and Urinary Excretion of Thiotepa and Its Metabolites. **B. E. Cohen, M. J. Egorin, E. A. Kohlhepp, J. Aisner, and P. L. Gutierrez.** University of Maryland Cancer Center, Baltimore

865 Phase I Study of Carboplatin (CBDCA) in Children With Cancer. **D. M. Bacha, B. Caparros-Sison, J. A. Allen, R. Walker, and C. T. C. Tan.** Memorial Sloan-Kettering Cancer Center, New York, New York

871 Trial of High-Dose Cytarabine, Cyclophosphamide, Total-Body Irradiation, and Autologous Marrow Transplantation for Refractory Lymphoma. **J. O. Armitage, R. D. Gingrich, L. W. Klassen, P. J. Bierman, P. P. Kumar, D. D. Weisenburger, and D. M. Smith.** University of Nebraska Medical Center, Omaha

877 Pharmacokinetics and Immediate Effects of High-Dose Carmustine in Man. **W. D. Henner, W. P. Peters, J. P. Eder, K. Antman, L. Schnipper, and E. Frei, III.** Dana-Farber Cancer Institute, Boston, Massachusetts

881 Toxicity of Oral *N*-Methylformamide in Three Phase II Trials: A Report From the National Cancer Institute of Canada Clinical Trials Group. **E. A. Eisenhauer, B. H. Weinerman, I. Kerr, and I. Quirt.** National Cancer Institute of Canada Clinical Trials Group

REVIEW ARTICLE

885 Aminothiadiazole: An Antineoplastic Thiadiazole. **P. J. O'Dwyer, B. H. Wagner, J. A. Stewart, and B. Leyland-Jones.** National Cancer Institute, Bethesda, Maryland

BRIEF REPORTS

891 Etoposide and High-Dose Cisplatin in Good-Risk Patients With Advanced Squamous Cell Carcinoma and Adenocarcinoma of the Lung. **A. Ardizzoni, L. Canobbio, P. Pronzato, A. Rubagotti, M. De Palma, F. Baracco, J. Serrano, S. Serrano, B. Faravelli, M. Nosenzo, R. Felletti, and R. Rosso.** Istituto Nazionale per la Ricerca sul Cancro, Genova, Italy

893 Cisplatin Plus Etoposide as Second-Line Treatment in Advanced Ovarian Carcinoma. **M. De Lena, V. Lorusso, and S. Romito.** National Institute for Cancer Research, Bari, Italy

897 Treatment of Advanced Gastric Carcinoma With Mitomycin and Doxorubicin. **D. Dalley, C. Erlichman, and S. Fine.** St. Vincent's Hospital, Sydney, Australia

899 High-Dose Mitoxantrone in Metastatic Breast Cancer: A Phase I-II Trial. **J. M. Leiby, D. V. Unverfurth, and J. A. Neidhart.** Ohio State University and Ohio State University Comprehensive Cancer Center, Columbus

903 Plasma Pharmacokinetics of 5-FU Given by Continuous Infusion With Allopurinol. **C. Erlichman, S. Fine, and T. Elhakim.** University of Toronto, The Princess Margaret Hospital, Ontario, Canada

905 Lhermitte's Sign as a Complication of Cisplatin-Containing Chemotherapy for Testicular Cancer. **R. Eeles, D. M. Tait, and M. J. Peckham.** Institute of Cancer Research and The Royal Marsden Hospital, Sutton, Surrey, England

909 Phase II Study of Cisplatin, 5-FU, and Allopurinol in Advanced Esophageal Cancer. **P. De Besi, V. C. Sileni, L. Salvagno, C. Tremolada, G. Cartei, V. Fosser, A. Paccagnella, A. Peracchia, and M. Fiorentino.** Ospedale Civile, Padova, Italy

CLINICAL TRIALS SUMMARIES

911 Phase II Evaluation of Oral Idarubicin (4-Demethoxydaunorubicin) in Patients With Disseminated Malignant Melanoma. **M. Lopez, L. Di Lauro, P. Papaldo, F. Ganzina, N. Di Pietro, and B. Lazzaro.** National Cancer Institute Regina Elena, Rome, Italy

913 Guanazole in the Treatment of Advanced Bronchogenic Carcinoma: A Pilot Study of the Southeastern Cancer Study Group. **S. Krauss, L. E. Broder, and R. Birch.** Southeastern Cancer Study Group

915 Treatment of Advanced Adenocarcinoma of the Kidney With ICRF-187: A Southeastern Cancer Study Group Trial. **L. H. Brubaker, C. L. Vogel, L. H. Einhorn, and R. Birch.** Southeastern Cancer Study Group

917 Phase II Eastern Cooperative Oncology Group Study of Spirogermanium in Previously Treated Lymphoma. **L. Boros, A. A. Tsiatis, R. S. Neiman, R. B. Mann, and J. H. Glick.** Eastern Cooperative Oncology Group

919 Ifosfamide Plus *N*-Acetylcysteine in the Treatment of Small Cell and Non-Small Cell Carcinoma of the Lung: A Southeastern Cancer Study Group Trial. **P. J. Loehrer, Sr, R. Birch, B. S. Kramer, and L. H. Einhorn.** Southeastern Cancer Study Group

921 Phase II Evaluation of Mitoxantrone in Advanced Renal Cell Carcinoma: A Southeastern Cancer Study Group Trial. **R. A. Gams, O. Nelson, and R. Birch.** Southeastern Cancer Study Group

923 Tamoxifen in the Treatment of Refractory Adenocarcinoma of the Cervix. **J. J. Kavanagh, J. T. Wharton, and F. Rutledge.** H. Lee Moffitt Hospital and Cancer Research Institute, University of South Florida, Tampa

925 Phase II Evaluation of Mitolactol in Squamous Cell Carcinoma of the Head and Neck: A Southeastern Cancer Study Group Trial. **M. S. McHale, E. Velez-Garcia, O. Nelson, S. D. Williams, W. Maddox, and R. Birch.** Southeastern Cancer Study Group

927 Combination Chemotherapy With Dacarbazine and Vindesine in the Treatment of Metastatic Malignant Melanoma. **D. A. Vorobiof, R. Sarli, and G. Falkson.** University of Pretoria and H. F. Verwoerd Hospital, Republic of South Africa

929 Weekly Mitoxantrone in the Treatment of Advanced Pancreatic Carcinoma: A Southeastern Cancer Study Group Trial. **P. A. DeSimone, R. Gams, and A. Bartolucci.** Southeastern Cancer Study Group

931 Limited Activity of Carboplatin in Malignant Lymphoma. **L. Danieu and R. P. Warrell, Jr.** Memorial Sloan-Kettering Cancer Center, New York, New York

LETTERS

933 High Incidence of Veno-occlusive Disease Related to Indicine *N*-Oxide in the Treatment of Refractory Adult Acute Leukemia. **E. F. Winton and P. A. McCue.** Emory University, Atlanta, Georgia

934 Effect of Diphenhydramine on the Antiemetic Action of Metoclopramide Against Cisplatin-Induced Emesis in Dogs. **W. L. Smith, C. B. Jackson, L. F. Sancilio, and R. S. Alphin.** A. H. Robins Research Laboratories, Richmond, Virginia

935 Treatment of Refractory Acute Lymphoblastic Leukemia With Teniposide and Cytarabine. **B. A. Kamen, J. Casper, S. Lauer, B. M. Camitta, and J. S. Holcenberg.** The University of Texas Health Science Center, Dallas

936 Destruction of Both Extensive Local Recurrent and Primary Breast Cancer With Topical Aqueous 5-FU: A Clinical Observation. **J. I. Zweig and B. Kabakow.** Beth Israel Medical Center, New York, New York

937 Rectal Administration of Medroxyprogesterone Acetate in Cancer Patients: A Pharmacokinetic Study. **F. Pannuti, B. Vancini, R. Comparsi, E. Strocchi, and C. M. Camaggi.** Ospedale S. Orsola-Malpighi, Bologna, Italy

939 ANNOUNCEMENTS

Doxorubicin, Ifosfamide, and Dacarbazine (AID) With Mesna Uroprotection for Advanced Untreated Sarcoma: A Phase I Study[1]

Anthony D. Elias* and Karen H. Antman[2,3]

The regimen of doxorubicin (DOX), ifosfamide (IFF), and dacarbazine (DTIC) (AID) for previously untreated inoperable or metastatic sarcoma has acceptable toxicity with significant activity. Twenty patients received 79 courses of DOX (60–75 mg/m^2) with or without DTIC (900 mg/m^2) by continuous infusion over 72 hours with escalating doses of IFF and mesna uroprotection. Nineteen patients were evaluable for toxicity. Myelosuppression was dose-limiting. The maximum tolerated dose was DOX at 60 mg/m^2, DTIC at 900 mg/m^2, and IFF at 7500 mg/m^2 per course. Of the 79 courses analyzed, 33 (42%) resulted in wbc counts $< 1000/\mu l$; 14 (18%) were complicated by fever and neutropenia, and three by sepsis. There were no toxic deaths. Relative platelet sparing was observed and nadirs were brief. In contrast to bolus-dose DTIC divided over 5 days, DTIC by continuous infusion did not add significantly to gastrointestinal toxicity. Nausea and vomiting was well controlled by antiemetics. Mucositis occurred sporadically. Unlike our phase II study of IFF alone, no CNS or renal toxicity was observed. No cardiac toxicity was encountered, although only four patients have received > 450 mg/m^2 of cumulative DOX. One episode of DOX extravasation occurred despite a long iv line that extended to the axilla. No serious tissue damage was observed, perhaps due to the dilute solutions of DOX used. Partial responses were seen in eight of 18 evaluable patients (44%) and in six of 11 patients at or near the phase II level. Two additional patients with minimal response have continued tumor regression. The median number of courses before partial response was four (range, one to five). The median duration of response has not been reached (3+ to 10+ months). An inoperable primary has been rendered surgically resectable in one patient. Activity in previously untreated sarcomas should be further evaluated in a randomized phase III study against a standard DOX-containing combination. [Cancer Treat Rep 70:827–833, 1986]

Effective chemotherapy for sarcomas has been limited by a lack of active drugs as well as the toxicity of therapy. A commonly used combination regimen includes cyclophosphamide (CPA), doxorubicin (DOX), and dacarbazine (DTIC). Despite higher response rates with two- or three-drug combinations, significant gastrointestinal, cardiac, and infectious complications have led some to advocate single-agent DOX as the standard of treatment. Recently, experience comparing continuous-infusion and bolus-dose DOX has demonstrated reduced cardiac toxicity while maintaining activity in breast carcinoma and sarcoma (1-3).

Ifosfamide (IFF), an analog of CPA, has now been extensively tested in European (4-13) and American (14-16) phase II trials in previously treated sarcoma and has shown a 35% response rate (range, 23%–78%) as a single agent (table 1). We have recently observed a response rate of 36% in 28 previously treated patients (14). A randomized British/Canadian study comparing lower doses of IFF with CPA suggested greater efficacy and more acceptable toxicity with IFF (17), confirming the findings in animal studies (18). IFF, like CPA, requires activation in the liver. The metabolic activation of IFF proceeds at half the rate of CPA; thus, the dechloroethylation reaction which produces inactive metabolites is a more important pathway for IFF (19,20). Animal studies and phase I and II trials in human lymphoma, small cell lung cancer, and sarcoma have shown a lack of cross-resistance with CPA, less myelosuppression, and more frequent hemorrhagic cystitis (14-16,21-26).

Mesna, a sulfhydryl uroprotector, has been reported

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[1] Received Aug 16, 1985; revised Jan 21, 1986; accepted Jan 30, 1986.

[2] Division of Medicine, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA.

[3] We thank Theresa Podedworny for careful preparation of this manuscript.

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TABLE 1.—Efficacy of IFF in previously treated sarcomas in order of dose delivered/course*

Ref No. (author)	IFF dose (g/m^2)	Schedule	Dose/course	Concurrent chemotherapeutic agents	Total No. of patients	Response†			Sarcoma‡
						CR	PR	Total (%)	
4 (Klein et al)	2.5–3.5	Daily × 5	12.5–17.5		11	1	4	45	STS§
5,6 (Czownicki and Utracka-Hutka)	1.25–2.5¶	Daily × 5	12.5		13	1	4	38	STS
7 (Scheulen et al)	2.5¶	Daily × 5	12.5		6		1	16	OGS
					6	1		16	ES
					16	2	3	31	STS
8 (Marti et al)	1.8	Daily × 5	9.0		18	2	4	33	OGS
15 (Magrath et al)	1.8	Daily × 5	9.0		18	0	4	22	STS**
					18	0	7	39	ES
					14	0	2	14	OGS
9 (Bierbaum et al)	1.65	Daily × 5	8.25	Cisplatin	16	3	5	50	STS§
					4	1	0	25	OGS
					1	1	0		ES
14 (Antman et al)	2.0–2.5	Daily × 4	8.0–10.0		28	0	10	36	STS**
					9	0	0	0	Pediatric STS
10 (Stuart-Harris et al)	5 or 8	Over 24 hrs	8.0		40	6	9	38	STS
16 (Pratt et al)	1.6	Daily × 5	8.0		24	1	7	33	Pediatric STS**
11,12 (Wellens et al)	1.5	Daily × 5	7.5	Etoposide	13	2	1	23	STS
13 (deKraker and Voute)	3.0	Daily × 2	6.0	Vincristine	7	2	5	78	STS
					4	2	1	75	OGS

**Modified from* ref 14.
†Values = No. of patients. CR = complete response; PR = partial response.
‡STS = soft tissue sarcoma; OGS = osteogenic sarcoma; and ES = Ewing's sarcoma.
§All CYVADIC (CPA, vincristine, DOX, and DTIC) failures.
¶Calculated from mg/kg as dose in g/kg × 70/1.7 $m^2 = g/m^2$.
**No difference in response between patients with prior treatment and no prior treatment with CPA.

to prevent hemorrhagic cystitis and to allow administration of larger doses of IFF. Inactive as the dimesna form in the circulation, mesna is reactivated in the kidney, and thus does not seem to interfere with the systemic antitumor effect of IFF.

We report the results of a phase I study in previously untreated metastatic or unresectable sarcoma using the combination of IFF with mesna uroprotection, and continuous-infusion DOX and DTIC.

METHODS

Eligibility criteria included: measurable histologically confirmed metastatic or inoperable sarcoma, performance score of 0–2 (< 50% of waking hours in bed), no prior chemotherapy, and normal end-organ function (creatinine < 1.5 mg/dl, bilirubin < 1.5 mg/dl, and no clinically evident congestive heart failure). IFF and mesna were supplied by the National Cancer Institute, Bethesda, MD. A temporary percutaneous or semipermanent surgically placed central venous silastic catheter was required for infusion of DOX. DTIC and DOX were mixed in 2 L of normal saline and infused at 100 ml/hr. The infusion was interrupted for 4 hours each morning to administer IFF at 100 ml/hr if a daily urinalysis detected < 50 rbcs per high-power field. Mesna, at 20% of the IFF dose, was given over 30 minutes 15 minutes prior to IFF, then every 4 hours for five doses after each dose of IFF by separate iv. Diuretics were used to offset the antidiuretic effect of IFF to maintain a urinary output of ≥ 100 ml/hr.

Subsequent courses of chemotherapy were repeated every 21 days, but were delayed by 1 week if the wbc and platelet counts were < 3000 and 100,000/μl, respectively. IFF was held for creatinine > 2.0 mg/dl. IFF and DOX were adjusted for hyperbilirubinemia (bilirubin of 1.5–2.9 mg/dl, 50% dose; 3.0–5.0 mg/dl, 25% dose; > 5.0 mg/dl, none). Cardiac function was assessed by radionuclide ventriculogram after approximately 400 mg/m^2 of DOX and then every two cycles to a cumulative DOX dose of 600 mg/m^2. If the patient continued to benefit, DOX was discontinued and IFF, mesna, and DTIC were continued with dose escalation of IFF to maintain a wbc count nadir of 1.0–1.5 × $10^3/\mu l$.

Review of the phase II IFF trials suggested a dose-response curve with higher response rates achieved at > 6 g/m^2/course. Therefore, DTIC was deleted from the regimen after the first five patients to attempt to allow further IFF escalation. No significant increase was possible; thus, the dose of DOX was reduced from 75 to 60 mg/m^2/course after ten patients were treated. This dose reduction allowed further dose escalation of IFF from 6.0 to 7.5 g/m^2/course with comparable hematologic toxicity. DTIC was reinstituted for the eighteenth patient. The phase II dose level was designed to produce a wbc count nadir of 0.5–1.5 × $10^3/\mu l$. A minimum of three courses were given per dose level until grade 4 hematologic toxicity was observed, after which in individual patients, IFF was increased by 0.5 g/m^2/day if the wbc count nadir was > 1500/μl, and was decreased by the same amount if the wbc count nadir was ≤ 500/μl for other grade 4 toxicity.

Standard response criteria were used (27). Partial

TABLE 2.—Patient entry characteristics

Characteristic	No. of patients
Entered	20
Median age in yrs (range): 51 (24–72)	
Sex	
Male	10
Female	10
Evaluable for toxicity	19*
Evaluable for response	18†

*1 patient was ineligible for toxicity due to a bilirubin of 3.2 mg/dl.

†1 patient was withdrawn with stable disease on Day 21 due to *Aspergillus* sepsis.

response was defined as a $> 50\%$ reduction in the product of perpendicular diameters of all measurable lesions for > 4 weeks. Tumor measurements were required at least every two cycles. Duration of response was calculated from the initiation of chemotherapy to the documentation of disease progression.

RESULTS

Twenty patients were entered between August 28, 1984, and April 12, 1985 (tables 2 and 3). Patients ranged in age from 24 to 72 years (median, 51). One patient had not responded to one prior course of CPA/vincristine/dactinomycin for a mistaken diagnosis of rhabdomyosarcoma; all others were untreated. One patient was ineligible due to a bilirubin of 3.2 mg/dl. Thus, 19 patients were evaluable for toxicity. Eighteen patients were evaluable for response; one was withdrawn due to poor performance status and systemic *Aspergillus* infection after one cycle.

Toxicity is summarized for 79 courses (and separately for the 25 courses at or near the phase II level; table 4). Myelosuppression was dose-limiting, with wbc counts of $< 1000/\mu l$ observed in 33 of 79 (42%) courses, and relative platelet sparing and brief nadirs. Fever and neutropenia developed in 14 courses (18%), with three episodes of bacteremia and no toxic deaths. Nonhematologic toxicity was remarkably mild. With antiemetics, grade 3 nausea and vomiting occurred in three of 79 courses (one of these three courses included DTIC). Grade 3 mucositis occurred twice. Two episodes of hematuria occurred just after Foley placement and resolved while IFF was continued. Four patients have received 750, 525, 525, and 450 mg/m^2 cumulative dose of DOX with no evidence of cardiotoxicity. One DOX extravasation developed around a percutaneously placed temporary long line that extended to the axilla. No surgical debridement was required. Two Port-a-Caths disconnected, but no extravasation occurred. All patients developed alopecia. No renal tubular acidosis, hepatitis, or unusual somnolence was noted. The maximum tolerated dose was determined by the dose that would produce a wbc count nadir (Day 14) of $500–1500/\mu l$ and a platelet count nadir of $> 50{,}000/\mu l$. Dose level 8 (DOX at 60 mg/m^2, IFF at 7.5 g/m^2, and DTIC at 900 mg/m^2 per course) was chosen as the phase II dose level (table 4). At doses at or near this level, nine of 25 (36%) courses resulted in wbc count nadirs $< 1000/\mu l$, with four (16%) episodes of fever and neutropenia. Platelet count nadirs of $< 20{,}000–50{,}000/\mu l$ occurred in two courses (8%). Mucositis ($\geq$ grade 2) occurred in five of 25 courses (20%).

Preliminary response data are summarized in table 3. Of 18 evaluable patients, eight (44%) have had partial response despite suboptimal doses of drugs early in the study. Responses were obtained more frequently at higher dose levels (six partial responses of 11 patients at or near the phase II level). Partial responses became apparent after one to five cycles (median, four). The median duration of response has not yet been reached (3+ to 10+ months). Two patients developed symptomatic brain metastases while their systemic disease continued to respond to chemotherapy.

DISCUSSION

While standard combination chemotherapy in sarcomas proves to be more effective than single-agent chemotherapy in terms of response rates (28–31), its success in the treatment of sarcomas has been impaired by its high degree of gastrointestinal toxicity. Our findings suggest that the regimen of DOX, IFF, and DTIC (AID) has significant activity with acceptable toxicity.

Table 5 summarizes some of the experience evaluating single-agent versus combination chemotherapy in advanced sarcomas (28–39). The objective response rate is approximately 35% for the most active single agent, DOX (40), with a probable dose-response curve suggesting poor response rates at < 60 mg/m^2/course (41–43). From the IFF data in previously treated sarcoma, it was felt that a dose > 6.0 g/m^2/course was important, while maintaining a DOX dose of 60 mg/m^2/course to maximize response potential. DTIC is also active in sarcoma, with single-agent response of 17% (44). DTIC may be less nauseating when administered by continuous infusion and can be mixed with DOX safely. Combinations of DOX and DTIC have demonstrated superior response and survival statistics in two trials (28–30). However, the DTIC schedule used resulted in such toxicity that single-agent DOX was advocated as the treatment of choice for metastatic sarcoma by these authors. A nonrandomized series of Southwest Oncology Group studies also demonstrated improved responses with combination chemotherapy, although the addition of vincristine has never been shown to improve response rates (31,32,42). A Dana-Farber Cancer Institute study evaluated CPA, DOX, and DTIC and confirmed the high response rate reported by the Southwest Oncology Group, also noting some long-term survivors (34). DOX is less cardiotoxic when administered by continuous infusion than by bolus injection, but both methods appear equally effective in the treatment of breast cancer and sarcoma (1–3).

TABLE 3.—Patient charac

Patient No.*	Age (yrs)	Sex	Histologic diagnosis†	Grade	Location of primary	Performance status	Metastases present
1	67	F	lip	2	Retroperitoneal	1	
2	63	F	lei	3	Bowel	1	X
3	63	F	fib	3	Chest wall	0	
4	24	M	neu	3	Lung	1	
5	28	M	lei	3	Bowel	0	X
6	67	M	mfh	3	RVOT§	4	
7	43	M	lei	3	Thigh	1	X
8	43	M	syn	3	Thigh	1	
9	24	M	hpc	3	Paraspinal	1	X
10	68	F	neu	1	Abdomen	1	
11	46	M	lei	1	Bowel	1	X
12	49	M	lei	3	Bowel	1	X
13	66	F	lei	3	Uterus	1	X
14	30	F	lei	3	Lung	2	X
15	44	M	lei	3	Bowel	1	
16	70	F	rms	3	Bladder	2	X
17	51	M	neu	3	Mesentery	1	X
18	47	F	neu	1	Bowel	1	X
19	72	F	lei	2	Lung	2	X
20	58	F	lei	3	Abdomen	3	X

*Patient 2—withdrew after 2 cycles due to moderate nausea/vomiting; Patient 6—presyncopal
Patient 13—developed liver failure during first course from progressive disease; Patient 19—withdr
liver failure on Week 4.

†Lip = liposarcoma; lei = leiomyosarcoma; fib = fibrosarcoma; neu = neurosarcoma; mfh = malig
rhabdomyosarcoma.

‡PR = partial response; SD = stable disease; DP = disease progression; and UE = unevaluable.

§RVOT = right ventricular outflow tract.

nt characteristics by response

stases sent	Inoperable primary	Starting dose DOX (mg/m^2)	DTIC (mg/m^2)	IFF (g/m^2)	Response status‡	Duration of response (mos)	No. of cycles of chemotherapy
	X	75	900	3.0	PR	10+	10
X	X	75	900	3.0	PR	4	2
	X	75	900	4.5	PR	9+	9
	X	75	0	4.5	PR	8+	8
X	X	75	0	4.5	PR	4	7
	X	60	0	6.0	PR	5+	6
X		60	0	6.0	PR	4+	5
	X	60	900	7.5	PR	3+	4
X	X	60	0	6.0	SD	5	4
	X	60	0	6.0	SD	4+	6
X		60	900	7.5	SD	4+	4
X	X	75	900	4.5	DP		4
X		75	900	4.5	DP		2
X		75	0	4.5	DP		3
	X	75	0	4.5	DP		2
X		75	0	4.5	DP		2
X	X	60	0	6.0	DP		2
X		60	900	7.5	DP		3
X	X	60	0	6.0	UE		1
X	X	60	0	6.0	UE		1

yncopal and in severe right heart failure from a 40-mm gradient across the pulmonary outflow tract; —withdrew after 1 cycle due to *Aspergillus* sepsis; Patient 20—ineligible (bilirubin of 3.2 mg/dl), died of

n = malignant fibrous histiocytoma; syn = synovial cell sarcoma; hpc = hemangiopericytoma; and rms =

aluable.

TABLE 4.—Toxicity by dose level

	Dose level											
	1	2*	3	4	5	6*	7*	8†	9*	10*	Total	At or near phase II level‡
DOX (mg/m^2)	75	60	75	75	60	75	60	60	75	60		
IFF (g/m^2)	3.0	4.5	4.5	4.5	6.0	6.0	7.5	7.5	7.5	9.0		
DTIC (mg/m^2)	900	0	0	900	0	0	0	900	0	0		
No. of courses (%)	3	3	9	19	20	4	9	9	2	1	79	25
Toxic effects§												
Nausea and vomiting (grade 3)	0	1	1	1	0	0	0	0	0	0	3 (4)	0
Wbc count ($\times 10^3$/mm^3)												
< 1.0	0	2	4	10	8	0	3	5	0	1	33 (42)	9 (36)
< 0.5	0	1	3	2	4	0	0	3	0	1	14 (18)	4 (16)
Platelet count ($\times 10^3$/mm^3)												
< 100	0	0	2	13	4	0	0	3	1	0	23 (29)	4 (16)
20-50	0	0	1	5	2	0	0	2	0	0	10 (13)	2 (8)
Fever and neutropenia	0	1	4	1	4	0	2	1	0	1	14 (18)	4 (16)
Mucositis ($\geq$ grade 2)	0	1	2	0	1	1	2	1	0	1	9 (11)	5 (20)

*These dosages were reached by dose modifications according to individual patient toxicity.

†Dose level 8 was the maximum tolerated dose level.

‡Dose levels 6-10 (%).

§Other toxic effects were as follows: 2 episodes of hematuria due to Foley placement for low urine output; 1 patient with atrial fibrillation, probably pre-existent, associated with hypothyroidism and liver failure; and 1 episode of DOX extravasation despite a peripheral line that extended to the axilla (tissue injury was mild).

TABLE 5.—Combination chemotherapy in sarcoma

Group (ref No.)*	Regimen†	No. of patients	No. of responses‡			Sarcoma§
			CR	PR	Total (%)	
ECOG (28)	AD	32	1	13	44	LMS
	A	34	1	5	18	
ECOG (29)	AD	95	4	25	30	STS
	A every 3 wks	93	6	12	19	
	A every wk	92	4	11	16	
ECOG (33)	A	66	4	14	27	STS + bone
	CAV	70	3	10	19	
	CAAdV	64	1	6	11	
SWOG (31)¶	A	49	2	13	31	STS
	AD	218	25	67	42	
	ADV	107	9	36	42	
	CADV	140	21	45	47	STS + bone
		125	21	42	50	STS only
SWOG (32)	CADV	221	31	84	52	STS
	CAAdV	224	27	63	40	
SWOG (42)	CADV	27	4	14	67	STS
	CADV + PEPA	24	8	9	71	
GOG (30)	A	80	5	8	16	Uterine
	AD	66	7	9	24	Sarcoma
DFCI (34)¶	CAD	23	4	9	56	STS
MSKCC (35)¶	CAVd	23	6	5	48	STS
(36)¶	CADM	36	4	5	25	STS
EORTC (37)	CADV	84	14	18	38	
	AD/alt CV	78	4	7	14	
SECSG (38)¶	CAM	45	2	15	38	
Bui et al (39)	CADV	60	4	25	48	

*ECOG = Eastern Cooperative Oncology Group; SWOG = Southwest Oncology Group; GOG = Gynecologic Oncology Group; DFCI = Dana-Farber Cancer Institute; MSKCC = Memorial Sloan-Kettering Cancer Center; EORTC = European Organization for Research on Treatment of Cancer; and SECSG = Southeastern Cancer Study Group.

†A = DOX; D = DTIC; C = CPA; V = vincristine; Ad = dactinomycin; PEPA = protective environment-prophylactic antibiotics; Vd = vindesine; M = methotrexate; and alt = alternating cycles with.

‡CR = complete response; PR = partial response.

§LMS = leiomyosarcoma; STS = soft tissue sarcoma.

¶Nonrandomized.

Hematologic toxicity with the AID regimen is similar to that reported for CYVADIC (CPA, vincristine, DOX, and DTIC) regimens, with about 25% of patients requiring antibiotics for fever and neutropenia (42). Hematologic suppression was usually brief; thus, severe infections were rare. No platelet support was needed. Nonhematologic toxicity of the AID regimen, however, was generally mild and no patients refused further therapy. In contrast, up to 18% of patients in one study using a standard chemotherapy combination (45) refused further therapy due to the nausea/vomiting, mucositis, and diarrhea frequently encountered in the CYVADIC regimens as well (39). In contrast to a schedule of bolus DTIC given five times a day, DTIC by continuous infusion (as used in this study) did not add significantly to gastrointestinal toxicity compared with patients who received DOX and IFF alone. Nausea and vomiting was well controlled with antiemetics. Mucositis, seen at the higher DOX dose of 75 mg/m^2, required dose reduction in only one patient. No CNS toxicity was observed in this study, but was prominent in a previous phase II study of single-agent IFF in refractory sarcomas (14). This may be due to the lower IFF dose per course as well as avoidance of heavily sedating antiemesis regimens. No significant cardiac toxicity was observed (but only four patients were at significant risk). A risk of congestive heart failure up to 14% has been reported for sarcoma regimens that utilize > 70 mg/m^2/course of DOX (46).

Thus, the AID regimen is a well-tolerated intensive regimen in previously untreated advanced sarcomas. The use of continuous-infusion DOX may allow a higher cumulative dose and may be a safer method for delivering high doses. As IFF is a potent single agent, DOX has been discontinued after 550-600 mg/m^2 cumulative dose and replaced by higher-dose IFF. One responder with an inoperable primary has been rendered surgically resectable. This regimen did not prevent the growth of brain metastases in two patients. The AID regimen should be further tested in a randomized phase III trial against a standard DOX-containing combination.

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Pharmacokinetics of Aclarubicin and Its Metabolites in Humans and Their Disposition in Blood Cells[1,2]

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The pharmacokinetics of aclarubicin, a new anthracycline antibiotic, was studied in five patients with acute leukemia or in L1210 cell suspension. Aclarubicin disappeared very rapidly from plasma and whole blood after administration at a dose of 20 mg per patient by iv bolus injection. The concentration of active metabolite M_1, on the other hand, increased for up to 2 or 4 hrs after administration and exceeded that of aclarubicin, and then remained at much higher concentrations than aclarubicin for up to 24 hrs after administration. In addition, the levels of aclarubicin and its metabolites in whole blood were much higher than the corresponding plasma levels in four of the patients. The drug concentrations in blood cells of 11 patients determined 4 hrs after administration showed a significant positive correlation with leukocyte counts. Moreover, the concentration of aclarubicin and its metabolites was found to be much higher in the leukocyte fraction than in the erythrocyte fraction in vivo and in vitro. These findings indicate that aclarubicin and its metabolites in blood cells were mainly accumulated in leukocytes. In the study of intracellular drug distribution in L1210 cells, the largest amount of aclarubicin was incorporated into the nuclear fraction. This suggests a close relationship between the pronounced drug accumulation in leukocytes and the high affinity of aclarubicin for DNA. [Cancer Treat Rep 70:835–841, 1986].

Aclarubicin is an anthracycline antibiotic isolated from the culture of *Streptomyces galilaeus* (1) and has potent antitumor activity against various types of experimental (1,2) and human (3) malignancies, especially hematopoietic neoplasms (4). This agent is characterized by a lower cardiac toxicity in hamsters (1,2,5) and by no mutagenicity in a *Salmonella* test (6) as compared with the commonly employed anthracyclines doxorubicin or daunorubicin.

As shown in figure 1, aclarubicin consists of a tetracyclic quinoid aglycone, aklavinone, and trisaccharides (rhodosamine, 2-deoxyfucose, and L-cinerulose A) attached to it via glycosidic linkage at C-7. The metabolism of aclarubicin has been well studied in vitro (7,8) and in animals (1,2), and the metabolites have been divided into two groups from their structures (fig 1). One group consists of the glycosides that retain antitumor activity, while the other group consists of aglycones that have no activity.

We have investigated the mechanism of the action of aclarubicin as compared with that of daunorubicin or doxorubicin (9). Aclarubicin was found to have the characteristics of anthracyclines, that is, high affinity for DNA and impairment of nucleic acid syntheses by the direct interaction with DNA. However, some differences in the mode of interaction with DNA between aclarubicin and daunorubicin or doxorubicin have been suggested.

In addition to the mechanism of action of the agent itself, its pharmacological property in patients is considered to be an important factor in determining its clinical efficacy. Therefore, we examined the pharmacokinetics of aclarubicin and its metabolites in humans and their disposition in blood cells by using high-

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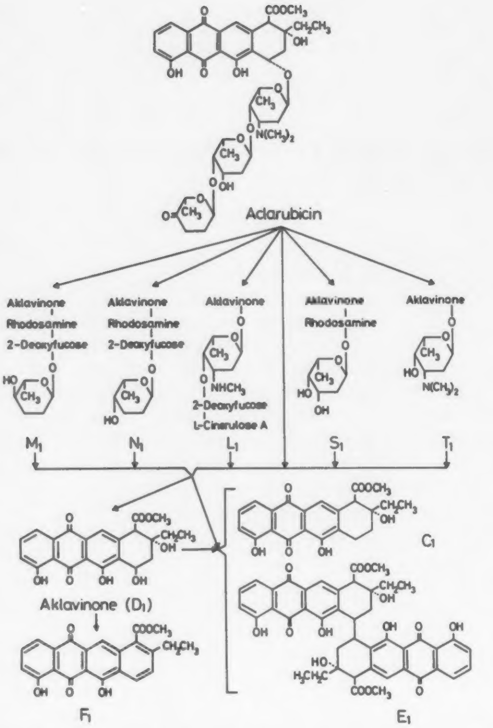

FIGURE 1.—Structure of aclarubicin and its metabolites as produced by proposed metabolic pathways.

performance liquid chromatography (HPLC) or radioassay to determine their concentrations.

MATERIALS AND METHODS

Chemicals

Aclarubicin and its labeled compound, [^{14}C]aclarubicin (1.22 mCi/mmol), were supplied by Sanraku Ocean Co, Tokyo, Japan. RPMI 1640 medium and fetal bovine serum were purchased from Nissui Pharmaceutical Co, Tokyo, Japan, and Flow Laboratories, McLean, VA, respectively. Other chemicals were commercial products.

Patients

All patients eligible for the pharmacological study were diagnosed as having acute leukemia by the usual hematological and clinical criteria; this was confirmed by bone marrow aspiration. One of the patients had abnormal hepatic function (GOT of 438 IU/L, GPT of 625 IU/L, and total bilirubin of 3.3 mg/dl) and another had abnormal renal function (BUN of 39 mg/dl and creatinine of 3.0 mg/dl).

Administration and Sample Collection

Aclarubicin (20 mg) was dissolved in 0.15 M NaCl and administered to 11 patients by iv bolus injection for 5 mins. Whole blood samples were collected in heparinized tubes at designated intervals for 24 hrs in five patients, and only after 4 hrs in the other six patients. Plasma and blood cellular fractions were separated by centrifugation of whole blood at 700 × *g* for 10 mins. Leukocyte and erythrocyte fractions were obtained by the dextran sedimentation procedure (10,11), and residual erythrocytes in the leukocyte fraction were lysed by exposure (at 37°C for 8 mins) to Tris-buffered ammonium chloride (12). Both leukocyte and erythrocyte fractions were then washed twice with Tris-buffered saline (0.15 M NaCl solution containing 8.6 mM Tris-HCl, pH 7.4).

Uptake by Human Blood Cells In Vitro

Each of the heparinized whole blood samples from four leukemic patients was incubated with 1.0 μg/ml of [^{14}C]aclarubicin at 37°C for 60 mins in a water-bath shaker. After the reaction was stopped by placing the test tubes in ice water, leukocyte and erythrocyte fractions were obtained by the dextran sedimentation procedure as described above. The protein content of each fraction was determined by the method of Lowry et al (13) with bovine albumin as the standard.

Preparation of L1210 Cell Suspension

L1210 cells were harvested from the peritoneal cavity of female BDF_1 mice 6 days after inoculation of 10^6 cells (the original L1210 cells and mice were kindly supplied by the Central Laboratories, Sankyo Co, Tokyo, Japan). The cell suspension was prepared as described previously (14). L1210 cells were finally suspended in RPMI 1640 medium supplemented with 20% fetal bovine serum to a final concentration of 10^7 cells/ml.

Subcellular Fractionation of L1210 Cells

L1210 cell suspension, 10^7 cells/ml, was incubated in the presence of 7.2 μg/ml of [^{14}C]aclarubicin at 37°C for 3 hrs in a water-bath shaker. The reaction was stopped by placing the test tubes in ice water. After washing out unincorporated [^{14}C]aclarubicin, the cell pellet was washed twice with a buffer containing 0.14 M NaCl, 3 mM KCl, and 10 mM sodium phosphate buffer, pH 7.25. Then, subcellular fractionation of the cells was performed as follows.

Nuclei were isolated by a modification of the method of Schandl (15) and Müllbacher and Ralph (16), and the other fractions were isolated using differential centrifugation by a modification of the method of Hogeboom (17) and DeChatelet et al (18). All operations were carried out at 0°C–4°C. Five times ten to the eighth cells were suspended in 6.0 ml of hypotonic buffer containing 3 mM $MgCl_2$, 2 mM EDTA, and 10 mM KH_2PO_4 (pH 7.7) for 10 mins, and then the cells were disrupted with

15 strokes in a tightly fitting Dounce homogenizer. An equal volume of buffer containing 22 mM glucose, 3 mM $CaCl_2$, 0.12 M NaCl, 0.12 M Tris-HCl (pH 8.0), and 2 mM EDTA was immediately added and the suspension was centrifuged at 1000 × *g* for 10 mins to recover a crude nuclear pellet. After being washed with a buffer containing 0.32 M sucrose, 2 mM $MgCl_2$, and 1 mM KH_2PO_4 (pH 6.8), the nuclear pellet was resuspended in the same buffer solution (nuclear fraction). Then, the supernatant from the first centrifugation was consecutively centrifuged at 10,000 × *g* for 15 mins, 27,000 × *g* for 30 mins, and 105,000 × *g* for 60 mins in a Hitachi RPS-40 swing rotor (Hitachi Koki Co, Tokyo, Japan) to obtain mitochondria, membrane, and microsome fractions as pellets, respectively. Each subcellular fraction was resuspended in 0.25 M sucrose. The resultant final supernatant was stored as the cytosol fraction. In our previous study, the effectiveness of all steps in this subcellular fractionation was confirmed by using electron microscopy (14). The protein content of each fraction was determined as described above.

HPLC Analysis

The concentrations of aclarubicin and its metabolites in the samples from in vivo study were determined by a modification of the method of Ogasawara et al (19) using HPLC. Five milliliters of 0.1 M phosphate buffer (KH_2PO_4-Na_2HPO_4, pH 7.0) and 18 ml of chloroform: methanol (1:1, vol/vol) were added to 1.0 ml of the sample. After gentle shaking for 10 mins, the mixture was centrifuged at 3000 rpm for 10 mins to obtain the organic layer. The aqueous layer was re-extracted with 9.0 ml of chloroform, and then the organic layers were combined together and evaporated to dryness. The residue was dissolved in 2.0 ml of chloroform:methanol and 1.0 ml of the internal standard solution (aclacinomycin B, 100 ng/ml in methanol). After further evaporation, the residue was finally redissolved in 0.2 ml of the mobile phase, acetonitrile:0.04 M ammonium formate buffer, pH 5.0 (1:1, vol/vol). The concentrations of aclarubicin and its metabolites in this sample were assayed by the following HPLC procedure.

A Shimadzu (Shimadzu Co, Kyoto, Japan) liquid chromatograph (model LC-3A) and an RF-500LC fluorescence detector were used with a Model C-RIA Chromatopak as both a recorder and an integrator. Aliquots (50 or 100 μl) of the sample were injected onto the column packed with reverse-phase μ Bondapak phenyl (Waters Associates, Milford, MA), and were eluted with the above-mentioned mobile phase at a flow rate of 1.3 ml/min. The analysis was carried out at 21°C ± 1°C. The quantities of aclarubicin and its metabolites detected by fluorometry using excitation and emission wavelengths of 435 and 505 nm, respectively, were determined by comparison of peak areas with their standards.

Assay of Radioactivity

The concentrations of aclarubicin and its metabolites in the samples from in vitro study were determined by their radioactivity. In [^{14}C]aclarubicin, the C-9 and methyl groups at the C-10 of aklavinone and at the C-3′ of rhodosamine were labeled biosynthetically by the incorporation of [1-^{14}C]propionate and [methyl-^{14}C] methionine (3). Therefore, all the metabolites shown in figure 1 could be detected by radioassay. Radioactivity

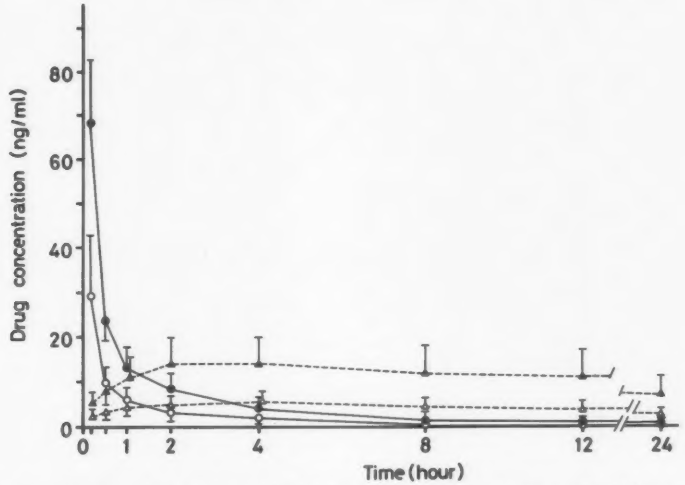

FIGURE 2.—Concentrations of aclarubicin and M_1 in four patients with acute leukemia. Aclarubicin was administered at a dose of 20 mg/patient by iv bolus injection for 5 mins. Drug concentrations in whole blood and plasma were determined by HPLC. ● = aclarubicin and ▲ = M_1 in whole blood. ○ = aclarubicin and △ = M_1 in plasma. Points = mean in 4 patients; bars = SD.

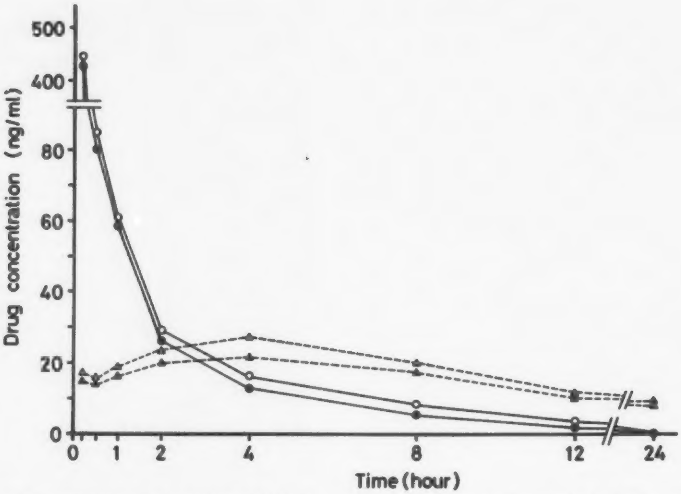

FIGURE 3.—Concentrations of aclarubicin and M_1 in a patient with acute leukemia who had abnormal hepatic function and proved to be resistant to aclarubicin. Drug administration and assays are described in the legend to fig 2. ● = aclarubicin and ▲ = M_1 in whole blood. ○ = aclarubicin and △ = M_1 in plasma.

was counted in scintillation fluid consisting of toluene and Triton X-100 (2:1, vol/vol), 2,5-diphenyloxazole (PPO; 4.0 g/L), and 1,4-bis-α-(5-phenyloxazolyl)-benzene (POPOP; 0.1 g/L) with an Isocap/300 liquid scintillation system (Searle Analytic Inc, Des Plaines, IL).

RESULTS

Pharmacokinetics of Aclarubicin and Its Metabolites in Patients With Acute Leukemia

The concentrations of aclarubicin and its metabolites in plasma and whole blood were determined in five patients up to 24 hrs after administration. Figure 2 shows the concentrations of aclarubicin and M_1 in four of the patients. Although there appeared to be considerable difference in concentration, especially shortly after administration, in each patient, the following common features were noted.

Aclarubicin itself disappeared very rapidly at first, and then slowly from plasma and whole blood. On the other hand, the concentration of M_1 increased up to 2 or 4 hrs after administration and exceeded that of aclarubicin, and then disappeared from plasma and whole blood more slowly than aclarubicin. There was no significant difference in drug disappearance between the patients with abnormal renal function and those with normal renal function. In addition, aclarubicin and M_1 levels in whole blood were much higher than the corresponding plasma levels throughout the determinations in all the patients except one who had abnormal hepatic function. The concentrations of aclarubicin and M_1 in this exceptional patient are shown in figure 3. The other metabolites, N_1, L_1, S_1, and D_1, were also detected in some patients, but their concentration was much lower than that of aclarubicin or M_1. The concentrations of these metabolites were also found to be much higher in whole blood than in plasma (data not shown).

The concentrations of aclarubicin and its metabolites in the blood cellular fraction determined in 11 patients 4 hrs after administration showed a significant positive correlation between blood cellular drug levels and leukocyte counts (fig. 4).

Intercellular Distribution of Aclarubicin and Its Metabolites

The concentrations of aclarubicin and its metabolites in leukocyte and erythrocyte fractions were determined in vivo and in vitro. In these studies, the drug concentration per milligram of protein was measured as an objective index of the affinity of the drugs for each fraction.

In the in vivo study, the concentrations in each fraction were determined in two patients 4 hrs after administration of aclarubicin. As shown in table 1, much higher concentrations were found in the leukocyte fraction than in the erythrocyte fraction. In the in vitro study, the concentration of aclarubicin incorporated into each fraction was determined after incubation of each of the heparinized whole blood samples from four patients with [^{14}C]aclarubicin. The results, shown in table 2, were in good agreement with those of the in vivo study.

Intracellular Distribution of Aclarubicin and Its Metabolites

A labeled L1210 cell suspension containing 5×10^8 cells was prepared after incubation with [^{14}C]aclarubicin at 37°C for 3 hrs. Then, subcellular fractionation

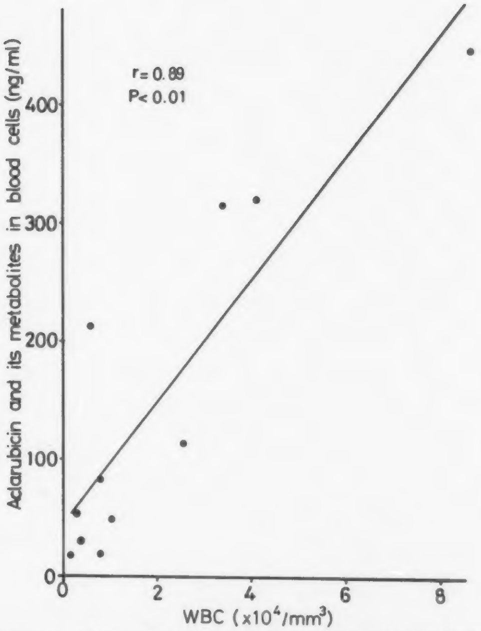

FIGURE 4.—Relationship between leukocyte counts and concentrations of aclarubicin and its metabolites in the blood cellular fraction. Drug concentrations were determined by HPLC in 11 patients 4 hrs after administration of aclarubicin at a dose of 20 mg/patient.

of the cells was performed. In the preliminary study using HPLC, aclarubicin was found to be > 75% of the total drug content in each fraction. As shown in table 3, the largest amount of radioactivity was found in the nuclear fraction, which means that aclarubicin and its metabolites were most prominently distributed in the nuclear fraction after incubation for 3 hrs. However, the concentration per milligram of protein was the highest in the cytosol fraction because of the much lesser content of protein in the cytosol fraction than in the nuclear fraction.

TABLE 1.—Intercellular distribution of aclarubicin and its metabolites in human blood cells in vivo*

Patient No.	Age (yrs)	Blood cells	Concentration in the cells (pg/mg of protein)†		
			Aclarubicin	M_1 and N_1	D_1
1	39	Wbc	1640	8330	1880
		Rbc	4	13	ND
2	70	Wbc	ND	5998	ND
		Rbc	ND	10	ND

*Leukocyte and erythrocyte fractions were obtained by the dextran sedimentation procedure 4 hrs after administration of aclarubicin at a dose of 20 mg/patient. Drug concentrations in each fraction were determined by HPLC.

†ND = not detected.

TABLE 2.—Intercellular distribution of aclarubicin incorporated into human blood cells in vitro*

Blood cells	Aclarubicin incorporated†	
	Concentration (ng/mg of protein)	Total contents (μg)
Wbc	733 ± 257	1.58 ± 0.14
Rbc	0.4 ± 0.6	0.10 ± 0.14

*Heparinized whole blood samples (2.5 ml) obtained from 4 leukemic patients were incubated with 1 μg/ml of [^{14}C]aclarubicin at 37°C for 60 mins. After incubation, leukocyte and erythrocyte fractions were obtained by the dextran sedimentation procedure. Drug concentration incorporated into each fraction was determined by its radioactivity.

†Values = mean ± SD.

DISCUSSION

The present pharmacokinetic study in humans revealed that aclarubicin disappeared very rapidly from whole blood and plasma after administration by iv bolus injection. On the other hand, the level of M_1 increased up to 2 or 4 hrs after administration and exceeded that of aclarubicin, and then M_1 remained at much higher concentrations than aclarubicin up to 24 hrs after administration. These results correspond well to the earlier reports (3,20). Since M_1 shows almost the same degree of antitumor activity against leukemia L1210 as aclarubicin (21), its pharmacokinetic property is considered to be important in the treatment with aclarubicin. In addition, the concentrations of aclarubicin and its metabolites in whole blood were found to be much higher than the corresponding plasma levels in all of the patients except one. This exceptional patient had abnormal hepatic function and proved to be resistant to aclarubicin. The higher plasma concentrations in this patient may be due to the impaired hepatic function, since it has been demonstrated in the pharmacokinetic study of doxorubicin that the patients with hepatic dysfunction have marked elevation and prolongation of plasma drug levels (22). Moreover, the uptake of daunorubicin has been reported to be significantly reduced in

TABLE 3.—Intracellular distribution of aclarubicin incorporated into L1210 cells*

Fraction	Aclarubicin incorporated†	
	Concentration (μg/mg of protein)	Total contents (μg)
Nucleus	9.98 ± 0.64	117.2 ± 15.4
Mitochondria	2.54 ± 0.52	2.5 ± 0.5
Membrane	4.56 ± 1.56	2.3 ± 0.6
Microsome	4.77 ± 1.22	1.2 ± 0.1
Cytosol	12.38 ± 0.17	82.7 ± 2.6

*L1210 cell suspension, 10^7 cells/ml, was incubated in the presence of 7.2 μg/ml of [^{14}C]aclarubicin at 37°C for 3 hrs. After incubation, subcellular fractionation of L1210 cells was performed. Drug concentration incorporated into each fraction was determined by its radioactivity.

†Values = mean ± SD from 3 experiments.

daunorubicin-resistant Ehrlich ascites cells (23,24). This finding may be applicable to aclarubicin-resistant cells, and so the lower concentrations in whole blood in this aclarubicin-resistant patient seem to result from the reduced uptake of aclarubicin by circulating leukemic cells.

Since the higher concentrations in whole blood than in plasma are apparently due to the pronounced drug accumulation in blood cells, the drug concentration in the blood cellular fraction was determined. There appeared to be a significant positive correlation between blood cellular drug levels and leukocyte counts (fig 4). Therefore, aclarubicin and its metabolites in blood cells were suggested to be accumulated mainly in the leukocytes. This was confirmed by the studies on intercellular drug distribution in vivo and in vitro (tables 1 and 2). We have reported that aclarubicin has high affinity for DNA and impairs nucleic acid syntheses by its interaction with DNA (9). This high affinity of aclarubicin for DNA may contribute to the pronounced drug accumulation in leukocytes that contain nuclear DNA. On the other hand, we found that N^4-behenoyl-1-β-D-arabinofuranosylcytosine (behenoyl-cytarabine), which is one of the newly synthesized derivatives of cytarabine, has low affinity for DNA and accumulates markedly in erythrocytes (14,25).

In the study on intracellular drug distribution in L1210 cells, the largest amount of aclarubicin was incorporated into the nuclear fraction after incubation for 3 hrs (table 3). This result confirms the abovementioned relation between the pronounced drug accumulation in leukocytes and the high affinity of aclarubicin for DNA. The concentration of incorporated aclarubicin per milligram of protein was the highest in the cytosol fraction (table 3). On the other hand, intracellular distribution of daunorubicin in L1210 cells prepared by the same method as this study using the subcellular fractionation method of Schneider (26) after incubation for 3 hrs (27) indicated that daunorubicin, as well as aclarubicin, is most prominently distributed in the nuclear fraction. However, daunorubicin shows a higher rate of drug distribution in the nuclear fraction than aclarubicin (daunorubicin, 77%; aclarubicin, 57%). Moreover, the concentration of incorporated daunorubicin per milligram of protein has been demonstrated to be approximately 2.5 times higher in the nuclear fraction than in the cytosol fraction. These differences seem to be more than those derived from the difference in the method used for subcellular fractionation of L1210 cells, since the ratio of protein content in the nuclear fraction to that in the cytosol fraction is comparable in both studies. Hence, aclarubicin is considered to have a somewhat different intracellular distribution pattern as compared with daunorubicin.

In our previous study, the binding of aclarubicin to DNA was suggested to be more labile than that of daunorubicin or doxorubicin (9). In addition, earlier reports have demonstrated that isolated L1210 cell nuclei accumulate more daunorubicin than aclarubicin (28) and that aclarubicin incorporated into the nuclei of Friend leukemia cells is rapidly released to the cytoplasm (29). These findings are compatible with our results that aclarubicin showed a lower rate of drug distribution in the nuclear fraction than daunorubicin. However, our results conflict with the reports that the distribution rates of aclarubicin and daunorubicin in the nuclear fraction were 78% and 70%, respectively, in Ehrlich ascites cells after incubation for 120 mins (24), and 91% and 62%, respectively, in cultured L1210 cells after incubation for 5 hrs (30). This discrepancy may be due to the difference in cells employed, in duration of incubation, or in subcellular fractionation method. Further comparative studies are required to resolve this discrepancy.

Finally, we demonstrated that the affinity of aclarubicin for DNA may reflect its accumulation in leukocytes. Therefore, in the pharmacokinetic studies of other drugs with high affinity for DNA, it is considered to be useful for an accurate analysis to determine the drug concentrations not only in plasma but in leukocytes.

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Phase II Trials of α-Difluoromethylornithine, an Inhibitor of Polyamine Synthesis, in Advanced Small Cell Lung Cancer and Colon Cancer[1,2,3]

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α-Difluoromethylornithine (DFMO), an irreversible inhibitor of ornithine decarboxylase, blocks polyamine synthesis and has demonstrated antitumor activity against small cell lung cancer and colon cancer in cell culture and animal tumor models. To evaluate clinical efficacy and further define toxic effects of this new agent, phase II trials of DFMO were performed in previously treated patients with advanced small cell lung cancer and previously untreated patients with metastatic colon cancer. Oral DFMO was administered at a dose of 2.25 g/m^2/day every 6 hours continuously to patients with small cell lung cancer. The same dose was given to patients with colon cancer but on a schedule of "3 weeks on, 1 week off" to avoid hearing loss. Evaluation of toxicity indicated that thrombocytopenia was seen only in patients receiving continuous DFMO who had received prior chemotherapy, while reversible hearing loss and gastrointestinal side effects occurred on both intermittent and continuous schedules in previously treated and untreated patients. [Cancer Treat Rep 70:843–845, 1986]

Interest in the role of polyamines in the growth processes of tumors has been heightened in recent years by the development of α-difluoromethylornithine (DFMO), an inhibitor of polyamine biosynthesis (1,2). In our laboratories, DFMO produced cell death in multiple lines of human small cell lung cancer and markedly inhibited the growth of established implants of cultured human small cell carcinoma in athymic mice (3). DFMO has also been shown to have antiproliferative action (alone and in combination with 5-FU) in a human colon adenocarcinoma cell line (4).

Based on these animal tumor model and cell culture results and on our phase I trial of DFMO (5), this phase II study of DFMO in advanced small cell lung cancer (SCLC) and colon cancer was conducted. This study was also designed to further define the toxic effects of DFMO in patients with and without prior exposure to cytotoxic chemotherapy.

MATERIALS AND METHODS

Twenty-seven patients with advanced SCLC who had received an average of 1.5 prior chemotherapy regimens (range, one to three) and 14 previously untreated patients with metastatic colon cancer were entered in this study between September 1982 and October 1984. Other eligibility criteria included measurable or evaluable disease; Eastern Cooperative Oncology Group (ECOG) performance status of 0–2; and adequate bone marrow, liver, and renal functions. Patients with SCLC must have had no chemotherapy for at least 3 weeks prior to entry. Patients with brain metastases or prior radiotherapy to present areas of measurable or evaluable disease were excluded. Informed written consent was required.

Oral DFMO was administered at a dose of 2.25 g/m^2 every 6 hours continuously to patients with SCLC. The same dose was given to patients with colon cancer but on a schedule of "3 weeks on, 1 week off" to avoid hearing loss. Dose adjustments were planned for gastrointestinal toxicity, auditory toxicity, and thrombocytopenia.

Standard ECOG criteria for solid tumor response and toxicity were used. In addition to the standard monitoring, the patients did undergo audiometric examinations.

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RESULTS

Patient characteristics are shown in table 1. Three patients with SCLC were not evaluable for response because they received < 21 days of DFMO. Two of these patients refused further treatment and one died of intercurrent pneumonia. One patient with SCLC was not evaluable for response and toxicity because he had thrombocytopenia prior to starting DFMO and only received 3 days of the drug. One patient with colon cancer was not evaluable for response or toxicity because he developed small bowel obstruction after 7 days of DFMO.

One partial response (transient shrinkage of a supraclavicular node) and no complete responses were observed in the SCLC population. There were no objective responses in the patients with colon cancer. The patients with SCLC were in the DFMO study for an average of 34 days, while the patients with colon cancer were in the study for an average of 49 days. The median survival (measured from the first day of DFMO) was 62 days for the patients with SCLC and 220 days for the patients with colon cancer.

TABLE 1.—Patient characteristics*

Characteristic	SCLC	Colon cancer
Total	27	14
Adequate trial for assessing response	23 (85)	13 (93)
Evaluable for toxicity	26 (96)	13 (93)
Performance status		
0	0 (0)	3 (21)
1	17 (63)	8 (57)
2	10 (37)	3 (21)
Sex		
Male	13 (48)	11 (79)
Female	14 (52)	3 (21)
Race		
Black	3 (11)	3 (21)
White	24 (89)	11 (79)
Age (yrs)		
<50	1 (4)	2 (14)
50-69	24 (89)	11 (79)
70	2 (7)	1 (7)
Tumor measurability		
Evaluable	7 (26)	1 (7)
Measurable	20 (74)	13 (93)
Limited disease	6 (22)	0 (0)
Extensive disease	21 (78)	14 (100)
Average No. of prior chemotherapy regimens	1.5	—
Treatment complications		
Anemia†	10 (38)	4 (31)
Leukopenia‡	5 (19)	0 (0)
Thrombocytopenia§	11 (42)	0 (0)
Auditory	5 (19)	7 (54)
Nausea/vomiting	18 (69)	5 (38)
Diarrhea	14 (54)	7 (54)
Response		
Complete	0 (0)	0 (0)
Partial	1 (4)	0 (0)
No change	3 (13)	2 (15)
Disease progression	19 (83)	11 (85)

*Unless otherwise specified, values = No. of patients (%).

†Hematocrit < 28% (ECOG 2-3).

‡Wbc count < 3000/mm^3 (ECOG 2).

§Platelet count < 90,000/mm^3 (ECOG 2-4).

Hematologic toxic effects (ECOG 2) are shown in table 1. Thrombocytopenia occurred in 42% of the patients with SCLC and in none of the patients with colon cancer. The median platelet count nadir in patients with thrombocytopenia was 59,000/mm^3 (range, 17,000-80,000); there were no episodes of bleeding secondary to thrombocytopenia. The thrombocytopenia occurred at a median cumulative DFMO dose of 224 g/m^2 and generally resolved at a median time of 15 days (range, 2-23). Mild leukopenia was seen in 19% of patients with SCLC, with no wbc count < 2700/mm^3. Anemia occurred in 38% of the patients with SCLC and in 31% of the patients with colon cancer and resulted in transfusion in only ten.

Nonhematologic toxic effects are shown in table 1. Gastrointestinal symptoms were common but mild to moderate in intensity (ie, ECOG 1 or 2) and readily controlled in all patients with colon cancer but two. Subjective hearing loss (ie, high frequency loss) was perceived in ten of 12 patients with this side effect; mechanical amplification was required in three. Auditory toxicity occurred after a median cumulative DFMO dose of 380 g/m^2 (356 g/m^2 in patients with colon cancer and 393 g/m^2 in patients with SCLC) and was resolved in all patients at a median interval of 24 days (range, 7-61).

DISCUSSION

In this phase II study, oral DFMO at a dose of 2.25 g/m^2 every 6 hours was not effective antitumor therapy in previously treated patients with SCLC and in patients with advanced colon cancer who had had no prior chemotherapy. Although our prior phase I study indicated that the dose schedule of DFMO used in this phase II trial should result in the minimal levels of DFMO necessary to inhibit polyamine biosynthesis in vitro, we do not have data regarding the degree of polyamine inhibition achieved in small cell and colon cancer lesions in this study. Since higher plasma levels of DFMO with less gastrointestinal toxicity can be achieved by the iv route of administration (6), further clinical trials will test DFMO on an iv schedule and will also include measurements of the degree of polyamine inhibition in normal hematopoietic precursors in bone marrow as well as in tumor masses.

The major toxic effect of DFMO was the hearing loss which occurred on both the intermittent and continuous schedule and in patients with and without prior exposure to cytotoxic chemotherapy. Gastrointestinal side effects were also common. However, these toxic effects may represent an effect of the oral preparation on gastrointestinal mucosa, since studies of DFMO administered iv have demonstrated virtually no nausea, vomiting, or diarrhea (6).

The hematologic toxicity was generally mild and readily reversible upon cessation of the drug. Thrombocytopenia occurred in 42% of the patients with SCLC but in no patients with colon cancer. In this study, it is not possible to determine whether the difference in incidence of thrombocytopenia related to the lack of prior cytotoxic therapy or to the intermittent schedule used in patients with colon cancer.

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Phase I Study of Continuous-Infusion Cisplatin[1]

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Cisplatin was administered to 21 patients as a continuous infusion over 5 days. Doses of 20, 25, and 30 mg/m^2/day were studied. When administered in 3 L of normal saline/day, continuous-infusion cisplatin at a dose of 30 mg/m^2/day was associated with neuropathy in two of nine patients and creatinine elevations ($>$ 2 mg/dl) in four. No neurotoxicity or nephrotoxicity was observed at lower doses. Severe thrombocytopenia ($<$ 50,000/μl) was unrelated to dose and occurred in 19% of the courses. Leukopenia was minimal. Nausea and vomiting were easily controlled. No toxicity was seen in 38% of the courses. Nineteen patients were evaluable for response. Six patients achieved partial response (32%), five had minimal response (25%), and eight had progressive disease (42%) in this group. Seven patients had received cisplatin previously as a bolus treatment (20 mg/m^2/day for 5 days) and failed on chemotherapy. Among these patients there was one with partial response, three with minimal response, and three with progressive disease. It is concluded that continuous-infusion cisplatin, as given in this protocol, is well-tolerated at a dose of 25 mg/m^2/day and can result in responses in patients failing bolus cisplatin at high doses. [Cancer Treat Rep 70:847–850, 1986]

Cisplatin has significant chemotherapeutic activity against a broad spectrum of human tumors including testicular, ovarian, head and neck, bladder, and esophageal carcinomas (1). Although this chemotherapeutic activity appears to be dose-dependent, dose-limiting nephrotoxicity and neurotoxicity occur. A number of methods have been developed to prevent renal toxicity associated with cisplatin treatment. These include the rapid infusion of large volumes of normal saline to induce a chloride diuresis or treatment with daily small-dose boluses. Although reduced by such measures, renal impairment still represents a major limitation for long-term or high-dose therapy. Moreover, recent reports have indicated that neurotoxicity represents a significant morbidity with these high-dose regimens (2).

Early in vitro studies by Drewinko et al (3) demonstrated that continuous exposure of neoplastic cells to cisplatin could result in in vitro reduction of cell viability equivalent to short-term exposures with doses an order of magnitude greater than those used for continuous exposure. Furthermore, there have been a number of studies utilizing continuous-infusion cisplatin that suggest that toxicity could be lessened by delivering the drug in this manner (4,5).

In the present study cisplatin was administered as a continuous iv infusion at doses starting at 20 mg/m^2/day over 5 days. This dose was chosen because of previous studies which suggest that 30 mg/m^2/day for 5 days might be the tolerable treatment level (4). In addition, cisplatin was administered in a 0.9% NaCl solution freshly prepared every 8 hours to prevent in vitro inactivation of the drug which may occur upon reconstitution in solutions with lesser concentrations of chloride ions (6). The purposes of this study were to: (*a*) determine the toxicity of cisplatin given in this manner; and (*b*) demonstrate that continuous-infusion cisplatin could result in responses in patients who had previously received cisplatin by other dose schedules.

METHODS

Twenty-four patients were entered in this study. Patients were eligible if they had clinically and histologically documented evidence of advanced malignancies for which routine treatments were not or had not been effective. Before receiving continuous-infusion cisplatin, patients had to recover completely from major toxic effects induced by prior therapy. Previous cisplatin by

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other than continuous infusion did not preclude patients from study. Patients were required to have measurable disease, serum creatinine < 1.5 mg/dl, and creatinine clearance > 75 ml/minute. Patients were admitted to the hospital for the duration of infusion and followed on an ambulatory basis.

Treatment was begun by prehydration with 1 L of normal saline over 2 hours. The dose of cisplatin to be delivered per day was administered in 3 L of normal saline with one-third of the total dose being given in each liter of fluid over 8 hours. Treatment was continued for 5 consecutive days. If there was no decrease in renal function, if the wbc count was $> 3000/\mu l$, and if the platelet count was $> 100{,}000/\mu l$ on Days 21 and 28, the dose was increased by 5 mg/m^2/day. If the wbc count was $< 2000/\mu l$ and the platelet count was $< 50{,}000/\mu l$, the dose was decreased by 5 mg/m^2/day.

During the inpatient phase of treatment, daily serum creatinine, electrolytes, BUN, magnesium, and calcium were required. A 24-hour urine creatinine clearance was performed prior to each treatment course. Responses were measured utilizing standard criteria. Complete response was defined as the complete disappearance of all clinical detectable disease for ≥ 4 weeks. Partial response was defined as a $\geq 50\%$ reduction in the product of the largest perpendicular diameters of all clinically measured tumors. Minimal response was defined as a $> 25\%$ regression and a $< 50\%$ regression in the product of the largest perpendicular diameters of the measurable tumor. Tumor progression was defined as any increase in the products of the largest perpendicular diameters of the measurable tumor. Stable disease was defined as disease progression.

RESULTS

Patient Characteristics

Nineteen of 24 entered patients were evaluable for response; 21 were evaluable for toxicity (table 1). Five patients were inevaluable for response because of the following reasons: received no treatment (one patient); lost to follow-up (one); received concurrent methotrexate (one); and no measurable disease (two). Three patients were inevaluable for toxicity because of the following reasons: lost to follow-up (one patient); not treated (one); and received concurrent methotrexate (one). The mean age of the patients was 59 years, and the male to female ratio was 17:4. The median performance status was 2.5 in the 21 patients evaluable for toxicity. Of the patients evaluable for response, there were seven with lung carcinoma, six with squamous cell carcinoma of the head and neck, and six with miscellaneous tumors. Of these patients, seven had received previous cisplatin and nine had received other treatments. Of the seven patients who had received cisplatin previously, six had received it as treatment for squamous cell carcinoma of the head and neck at a dose of 100 mg/m^2 as a daily bolus of 20 mg/m^2 for 5 days.

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Entered	24
Evaluable for toxicity	21
Evaluable for response	19
Mean age in yrs (range): 59 (32–75)	
Male:female	17:4
Performance status*	21
0	4
1	1
2	8
3	5
4	3
Tumor	19
Head and neck carcinoma (squamous cell)	6
Small cell lung carcinoma	3
Non-small cell lung carcinoma	4
Miscellaneous	6
Previous therapy	21
Chemotherapy	16
Radiotherapy	16
None	2
Cisplatin	8

*Eastern Cooperative Oncology Group criteria.

Toxicity

Toxicity at each dose level is shown in table 2. Myelotoxicity was minimal, even at a dose of 30 mg/m^2/day. Toxicity did not appear to correlate with dose level achieved but appeared to be related to amount and duration of previous therapy. One significant infection occurred during leukopenia in a patient receiving 25 mg/m^2/day. Significant thrombocytopenia developed in 19% of the courses. One course was associated with significant bleeding during thrombocytopenia from a large carcinoma of the tongue which was regressing. The patient recovered from this while having a partial response to treatment. Creatinine elevations were only noted at a dose of 30 mg/m^2/day and occurred in four of nine patients receiving this dose (table 2). Transfusion requirements unrelated to hemorrhage during therapy occurred frequently. Notably, neurotoxicity occurred in two patients receiving the 30-mg/m^2/day dose after the second or third course, whereas no neurotoxicity was seen at 20 or 25 mg/m^2/day. Significant nausea and vomiting occurred (Grade III, Eastern Cooperative Oncology Group criteria) in two courses at 25 mg/m^2/day. Four of nine courses at 20 mg/m^2/day were associated with no toxicity, while eight of 20 courses at 25 mg/m^2/day were associated with no toxicity. Three of 13 courses at 30 mg/m^2/day were associated with no significant toxicity.

The median number of courses received by each patient was two. Seven patients received one course of chemotherapy, eight received two courses, five received three courses, and one received four courses. A total of

TABLE 2.—Toxicity of continuous-infusion cisplatin

Toxic effect	No. of courses 20 mg/m^2 ($n = 9$)*	 25 mg/m^2 ($n = 20$)*	 30 mg/m^2 ($n = 13$)*	 Total ($n = 42$)*
Wbc count				
$< 2000/\mu l$	0	2 (10%)	3 (23%)	5 (12%)
$< 1000/\mu l$	2 (22%)	1 (5%)	0	3 (7%)
Platelet count				
$< 100{,}000/\mu l$	2 (22%)	6 (30%)	4 (31%)	12 (29%)
$< 50{,}000/\mu l$	1 (11%)	5 (25%)	2 (15%)	8 (19%)
Creatinine > 2 mg/dl	0	0	4 (31%)	4 (10%)
Anemia	1 (11%)	4 (20%)	4 (31%)	9 (21%)
Neurotoxicity	0	0	2 (15%)	2 (5%)
Infection	0	1 (5%)	0	1 (2%)
Nausea and vomiting	0	2 (10%)	0	2 (5%)
None	4 (44%)	8 (40%)	3 (29%)	15 (36%)

*No. of courses at risk.

42 courses were given. Nine courses were given at 20 mg/m^2/day, 20 were given at 25 mg/m^2/day, and 13 were given at 30 mg/m^2/day.

Response

Of the 19 patients evaluable for response, there were six with partial response, five with minimal response, and eight with progressive disease (table 3). The median duration of response was 2 months, with a range of 1.5-4+ months. The median survival was 4 months, with a range of 2-19+ months. Six patients with squamous cell carcinoma of the head and neck were treated. All six patients had been treated previously with bolus cisplatin. One patient achieved partial response, three had minimal response, and two had progressive disease on this protocol (table 4). Among the patients with lung carcinoma, there were three with small cell carcinoma of the lung. All three patients had received previous chemotherapy with regimens not containing cisplatin. One patient achieved partial response, none had minimal response, and two had progressive disease. Of four patients with non-small cell carcinoma of the lung, none had received previous chemotherapy. There were two partial responses and two minimal responses. Among the miscellaneous tumors treated, two patients achieved partial response and four had progressive disease. Responses occurred in prostate (one response among one patient) and adenoidcystic (one response among one patient) carcinomas.

DISCUSSION

We have demonstrated that continuous-infusion cisplatin is effective chemotherapeutic treatment and that

TABLE 3.—Response to continuous-infusion cisplatin

Tumor	Partial response	Minimal response	Progressive disease	Total
Head and neck carcinoma	1	3	2	6
Small cell lung carcinoma	1	0	2	3
Non-small cell lung carcinoma	2	2	0	4
Miscellaneous	2	0	4	6
Total	6	5	8	19

TABLE 4.—Response to continuous-infusion cisplatin after failure with bolus treatment

Tumor	Partial response	Minimal response	Progressive disease	Total
Head and neck carcinoma	1	3	2	6
Miscellaneous	0	0	1	1
Total	1	3	3	7

this therapy can induce responses in patients who have received previous treatments with bolus cisplatin despite previous failure at these dose levels. Finally, this study demonstrates that while the toxicity associated with continuous-infusion cisplatin in this heavily treated population was significant, it is tolerable. Of particular note is the low incidence of neurotoxicity and nephrotoxicity present with these high doses of continuous-infusion treatment.

Studies by Drewinko et al (3) indicate that the continuous exposure of malignant cells to cisplatin in vitro can result in significantly greater reductions in colony formation and cell viability when compared to limited exposures to cisplatin at doses an order of magnitude greater. These data suggest that the cytotoxic and antitumor activity of cisplatin might be improved by increasing the duration of in vivo exposure of malignant cells to cisplatin.

In this report we show that continuous-infusion cisplatin can result in an antineoplastic effect in patients with tumors which have progressed on treatment with bolus cisplatin at high dose. These responses represent marginal improvements in terms of duration and extent in these aggressive neoplasms. Of equal importance is the lack of significant toxicity associated with this treatment. Nephrotoxicity did not occur at the 25-mg/m^2 dose level, and, overall, nausea and vomiting were easily controlled with antiemetics. Neurotoxicity was not detected at the dose of 25 $mg/m^2/day$ but did occur when the dose was increased to 30 $mg/m^2/day$. On the basis of the present study we feel that continuous-infusion cisplatin should be given at 25 $mg/m^2/day$ and suggest that there may be a threshold level at which neurotoxicity is observed in these patients. It would appear that it is important to determine if the in vivo pharmacokinetics of cisplatin given as a continuous infusion are altered when compared to bolus infusion. Comparative trials of continuous-infusion and bolus cisplatin in responsive tumors are indicated by the results of this trial.

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Intratumoral Injection of Thymidine: Enhancement of the Antitumor Activity and Incorporation of 5-Fluorouracil Into Tumor RNA in a Murine Tumor System[1,2]

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Intratumoral injection of thymidine (dThd) into mice bearing an Ehrlich ascites solid tumor in combination with an ip injection of 5-fluorouracil (FUra) was performed to investigate the following: (*a*) whether intratumoral injection of dThd would increase both the incorporation of FUra into tumor RNA and the antitumor activity of FUra as effectively as ip injection of dThd, and (*b*) whether intratumoral injection of dThd would have a selective advantage for increasing the incorporation of FUra into the tumor RNA, relative to normal tissue RNA. Pulse-labeling with [^{3}H]FUra at different times after dThd injection indicated that the most effective incorporation of FUra into the tumor RNA occurred with both intratumoral and ip injection of dThd, when FUra and dThd were given simultaneously. The amount of FUra-containing RNA [(FU)RNA] was dThd dose-dependent and reached a maximum at a dThd dose of 100 mg/kg, in the case of either intratumoral or ip injection. The maximal level with intratumoral injection of dThd was twofold higher than the level with ip injection and at least fivefold higher than the level achieved with FUra alone. In a time course study, the amount of (FU)RNA formed after intratumoral injection of dThd was comparable to the level obtained with ip injection, which was fourfold to sixfold greater than that seen with FUra alone. Furthermore, in the four normal organs (bone marrow, intestine, kidneys, and lungs) the amount of (FU)RNA formed after intratumoral injection of dThd was markedly lower than the level with ip injection. Assay of FUra degradation products revealed low levels of FUra catabolism in the tumor tissue compared to liver. In addition, therapy experiments showed that the antitumor activity of FUra was increased 17-fold by coadministration of dThd by either route. [Cancer Treat Rep 70:851-857, 1986]

5-Fluorouracil (FUra) is a widely used drug for cancer chemotherapy (1-3). In several experimental tumor model systems, the antitumor activity of FUra has been shown to be potentiated by systemically administered thymidine (dThd), probably through the latter's capacity to increase the incorporation of FUra into the RNA of tumor tissue (4-11). Such enhancing effects of FUra incorporation into RNA by dThd treatment also occur in in vitro systems (12), thereby suggesting a direct action of dThd on the tumor cells.

A persistent problem in cancer chemotherapy is the lack of selectivity of anticancer drugs. One approach to this problem would be to improve the selectivity by enhancing the local antitumor effects of drugs administered systemically. Therefore, we examined the local injection of dThd into tumor, with regard to its effect on the antitumor activity of FUra and the incorporation of FUra into the tumor RNA.

We found that dThd injected into the solid tumors formed from sc injection of Ehrlich ascites cells selectively increased the incorporation of ip administered FUra into tumor RNA as compared to normal tissues and potentiated the antitumor activity of FUra, without any increase in mortality.

MATERIALS AND METHODS

Chemicals.—dThd, orcinol, NADPH, and yeast RNA were obtained from Sigma; 2-fluoro-3-ureidopropinic acid (F-β-UPA) and 2-fluoro-alanine (F-β-Ala) from Taiho Pharmaceutical Co, Japan; and FUra from Hoff-

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mann-La Roche. [^{3}H]FUra (3.83 Ci/mmol, radiochemical purity was 97% by thin-layer chromatography in *n*-butanol saturated with water) and [^{14}C]FUra (55 mCi/mmol, radiochemical purity, 98%) were from Amersham [^{3}H]dThd (20 Ci/mmol) was from New England Nuclear.

Animals and tumor.—Adult female ddY mice (CLEA Japan), 8-9 weeks of age and weighing 28-32 g, were used. Ehrlich ascites tumor cells (1×10^7), obtained from Prof. Tsuneo Baba, Kyushu University, were transplanted into the dorsum sc. Food and water were provided ad libitum.

dThd concentrations in the tumor and various organs.—When the tumors reached 2 cm in diameter, 18-21 days after inoculation, dThd (100 mg/kg) plus [^{3}H]dThd (1.5 mCi/kg) were injected locally into the tumor or ip together with a concomitant ip administration of FUra (10 mg/kg). At various times, the animals were killed by cervical dislocation. The tumors and various normal organs, bone marrow, intestine, kidneys, liver, and lungs were homogenized, sonicated, and dissolved in 0.3 N KOH. A portion of the KOH solution was used to measure the radioactivity in a water-miscible liquid scintillator, Phase Combined System (PCS, Amersham). In the remaining portion, the protein content was quantified by a Bio Rad protein assay kit (Bio Rad Laboratories Japan KK, Tokyo). The results were expressed in counts per minute of [^{3}H]dThd equivalents per milligram of protein.

Measurement of the incorporation of FUra into RNA.—Either saline or various doses of dThd were injected into the tumor or peritoneal cavity with a concurrent administration of FUra (10 mg/kg) containing [^{3}H]FUra (3 mCi/kg). At designated times, the animals were killed and the tumors, intestine, kidneys, liver, and lungs were excised. The bone marrow was collected by flushing the femur with ice-cold water. The RNA of the tumor and each normal tissue was extracted according to the method of Schmidt and Thanhauser (13). Briefly, the tissues were homogenized in ice-cold water followed by the addition of trichloroacetic acid (TCA, finally 10%). The homogenate was centrifuged and the pellets were washed twice with ethanol:ether (3:1), dissolved in 0.3 N KOH, and maintained at 37°C for 20 hours to hydrolyze the RNA. The KOH solution was cooled, made up to 5% with TCA, and centrifuged. A portion of the RNA hydrolysate was used to count the radioactivity and the remaining portion was used for the quantification of RNA by the method of Schneider (14). The radioactivity in RNA was calculated in terms of nanograms of FUra per milligram of RNA, on the basis of specific activity of [^{3}H]FUra.

Assay of FUra degradation.—The catabolism of FUra in the liver and tumor was examined according to the methods of Ikenaka et al (15). Briefly, for preparation of enzyme solution, the liver and tumor were removed from mice, freeze-thawed twice, and homogenized in 0.25 M saccharose containing 2-mercaptoethanol and EDTA. For the FUra degradation assay, the reaction mixture consisting of NADPH, ATP, nicotinamide, potassium phosphate buffer (pH 7.6), [^{14}C]FUra (12.8 μCi/ml), and homogenate was incubated at 37°C for 10 minutes for liver or 60 minutes for tumor. The reaction was halted by adding $HClO_4$ at 4°C. The mixture was centrifuged and KOH was added to the supernatant. After standing for 5 minutes at 4°C, 10 μl of the supernatant was applied to a thin-layer chromatography plate with silica gel and developed with ethanol plus ammonium acetate. FUra was identified by measuring UV absorption, while F-β-Ala and F-β-UPA were identified with ninhydrin and Ehrlich reagent, respectively. Each spot was extracted with 4 M HCl, and the radioactivity was determined.

Chemotherapy.—Tumor-bearing mice were divided into seven groups ($n = 15$/group) based on differences in the injection sites and the combinations of dThd and FUra. Details on grouping are given in figure 1. The drugs were given on alternate days, starting when the

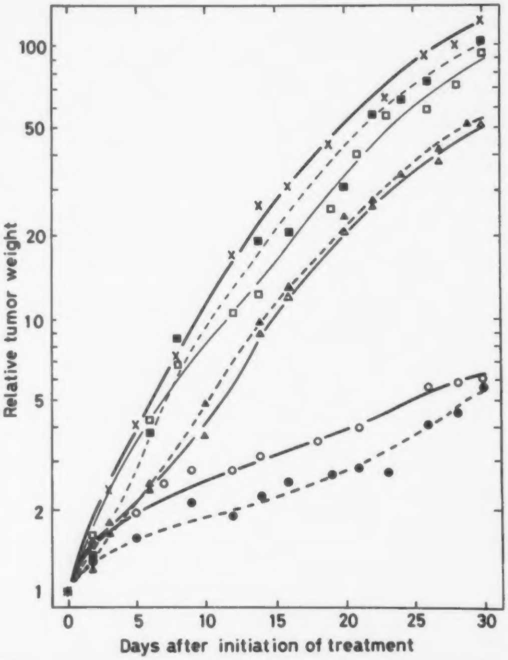

FIGURE 1.—Enhancement of therapeutic activity of ip administered FUra by intratumoral or ip injection of dThd. Tumor-bearing mice were divided into 7 groups, depending on the site of injection and the combination of dThd and FUra. The 15 mice in each group were treated on alternate days. Doses of dThd and FUra were 100 and 10 mg/kg, respectively, in any group given the drugs. Group 1 = intratumoral + ip of saline (X); Group 2 = intratumoral of dThd + ip of saline (■); Group 3 = ip of saline and dThd (□); Group 4 = intratumoral of saline + ip of FUra (▲); Group 5 = ip of saline and FUra (△); Group 6 = intratumoral of dThd + ip of FUra (○); and Group 7 = ip of dThd and FUra (●). Mean tumor weight at initiation of treatment was 108 ± 30 mg. Each point represents the mean of relative tumor weight in 15 mice.

mean diameter of the tumor was 6 mm and continuing throughout the course of the experiment. The chemotherapeutic effects were observed by suppression of the tumor growth and were determined by measuring the size of the tumor (longest axis, L; and shortest axis, S) with a caliper once per 2-3 days. Tumor mass was estimated according to the formula: tumor mass (mg) = L (mm) × $S^2/2$ (mm^2) (11). Student's t test was used for statistical evaluation of differences in tumor response between each treatment group.

RESULTS

The intratumoral radioactivity after [^{3}H]dThd administration either intratumorally or ip is compared in figure 2. When dThd was given intratumorally there was a much higher level of radioactivity (threefold to 30-fold) than was observed with ip injection, and this difference remained obvious for up to 3 hours after administration of dThd (fig 2, A). The radioactivity contained in the bone marrow, intestine, liver, and lungs was about twofold lower in the case of intratumoral injection than with ip injection of dThd; similar levels, however, were observed in the kidneys (fig 2, B-F).

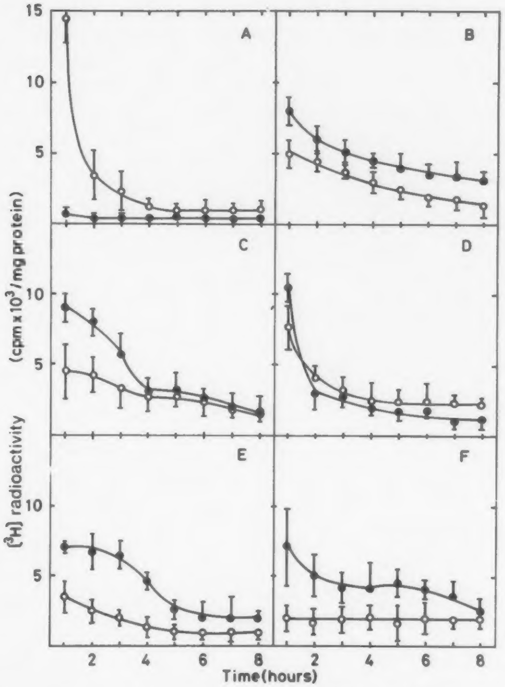

FIGURE 2.—Time course change of radioactivity in the tumor and various normal organs after [^{3}H]dThd administration. Tumor-bearing mice were treated by intratumoral (○) or ip (●) injection of dThd (100 mg/kg) + [^{3}H]dThd (3 mCi/kg), under conditions in which FUra (10 mg/kg) was given ip. At the indicated times, the tumor (A), bone marrow (B), intestine (C), kidneys (D), liver (E), and lungs (F) were removed and radioactivity due to [^{3}H]dThd, [^{3}H]dThd nucleotides, [^{3}H]dThdDNA, and [^{3}H]dThd catabolites in these tissues was measured and normalized to protein. Vertical bars are SE in determinations in tissues from 3 mice.

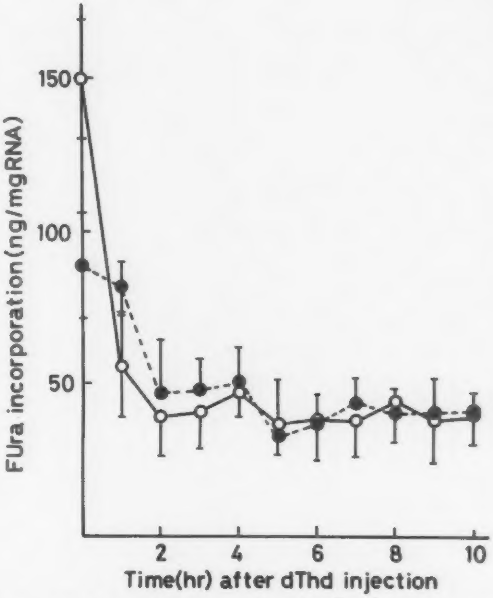

FIGURE 3.—Pulse-labeled incorporation of FUra into tumor RNA. Labeling was carried out for 1 hr with ip administration of FUra (10 mg/kg) + [^{3}H]FUra (3 mCi/kg) at the indicated times after dThd (100 mg/kg) injection into the tumor (○) or peritoneal cavity (●). Tumors were excised and radioactivity in the tumor RNA was measured. Bars are SE in 3 determinations.

The optimal time of administration of FUra after intratumoral or ip injection of dThd was determined by pulse-labeling for 1 hour with [^{3}H]FUra at different times after dThd injection (fig 3). The concurrent administration (at 0 time) of FUra and dThd into respective sites resulted in the highest incorporation in the case of both intratumoral and ip injection of dThd, compared with the levels at subsequent times. On the basis of these results, dThd and FUra were given simultaneously in subsequent experiments.

The effect of dThd dose on the incorporation of FUra into RNA is shown in figure 4. In case of both intratumoral and ip injection of dThd, the incorporation increased with the dose of dThd up to 100 mg/kg. Treatment with doses > 100 mg/kg both intratumorally and ip did not increase the levels of incorporation of FUra. The maximally stimulated incorporation of FUra into RNA with injection of dThd was at least sixfold higher than the level with saline and twofold higher than the level with ip injection of dThd after 1-hour exposure to the combination.

It was important to determine whether intratumoral injection of dThd would have a continuous effect on the incorporation of FUra into tumor RNA. Therefore, we

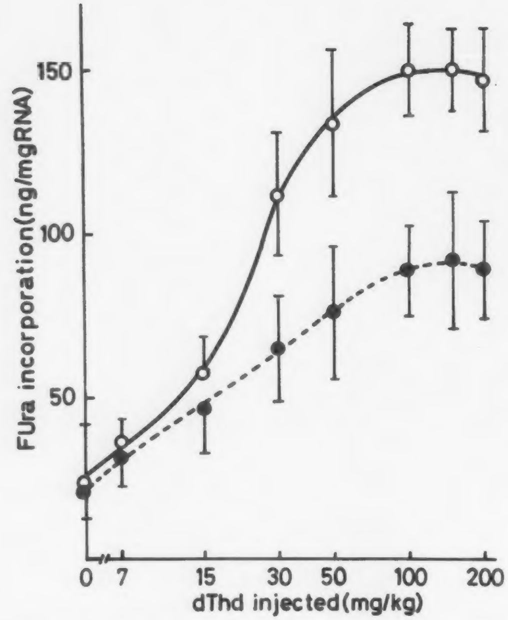

FIGURE 4.—Effect of the dose of dThd on the incorporation of FUra into tumor RNA. Various doses of dThd were injected into the tumor (○) or peritoneal cavity (●) together with ip administration of FUra (10 mg/kg) + [^{3}H]FUra (3 mCi/kg). After 1 hr, the tumors were excised and radioactivity in the RNA was measured. Bars are SE in 3 determinations.

examined the time course change of the incorporation of FUra into RNA after either intratumoral or ip dThd (fig 5). The amount of FUra incorporated into RNA [(FU)RNA] with intratumoral injection of dThd was maximum at 1 hour and remained essentially constant at least up to 8 hours, at a level threefold that seen in the control group. It should be noted that the increased (FU)RNA with intratumoral injection of dThd was comparable to the level with ip injection after 2 hours.

Since (FU)RNA is toxic to tissue cells, we assessed the potential for normal-tissue toxicity by measuring the effects of dThd on FUra incorporation into RNA of the bone marrow, intestine, kidneys, liver, and lungs after either intratumoral or ip injection of dThd and ip administration of FUra (fig 6). In the tumor (calculated from fig 5), intratumoral injection of dThd increased the amount of (FU)RNA by fourfold to sixfold over the control, while in various normal organs tested, the level was less than threefold over the control (fig 6, A). The ip administration of dThd increased the amount of (FU)RNA in the kidneys and lungs by sevenfold over the control, in contrast to having only a fourfold effect in the tumor (fig 6, B). After ip dThd in the bone marrow ($P < 0.05$ at 5 hours), intestine ($P < 0.05$ at 4 and 5 hours), kidneys ($P < 0.05$ at 2 and 3 hours), and lungs ($P < 0.05$ at 2-5 hours), there was a tendency toward higher amounts of (FU)RNA than those seen with intratumoral injection of the drug. In the liver, statistically significant differences of (FU)RNA levels between intratumoral and ip dThd were not noted (fig 6, A and B). Thus, intratumoral injection of dThd seems to offer a

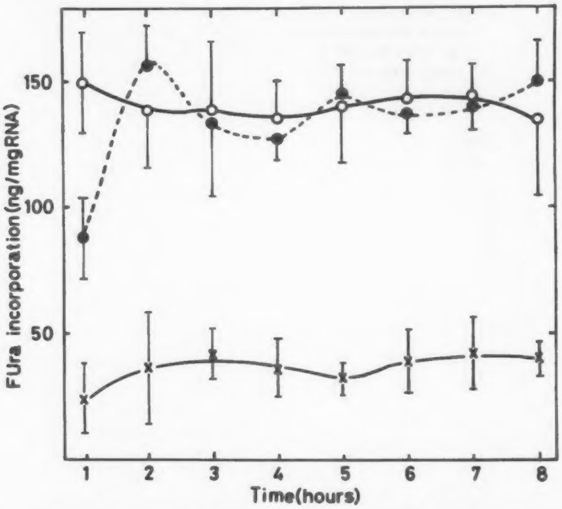

FIGURE 5.—Effect of dThd on the incorporation of ip administered FUra into tumor RNA. Tumor-bearing mice were given intratumoral injection of saline (X) or dThd (100 mg/kg) (○) or ip injection of dThd (100 mg/kg) (●) together with ip administration of FUra (10 mg/kg) + [^{3}H]FUra (3 mCi/kg). At the indicated times, animals were killed and the radioactivity incorporated into tumor RNA was determined and converted into nanograms, according to the specific activity of the labeled compound. Bars are SE in 3 determinations.

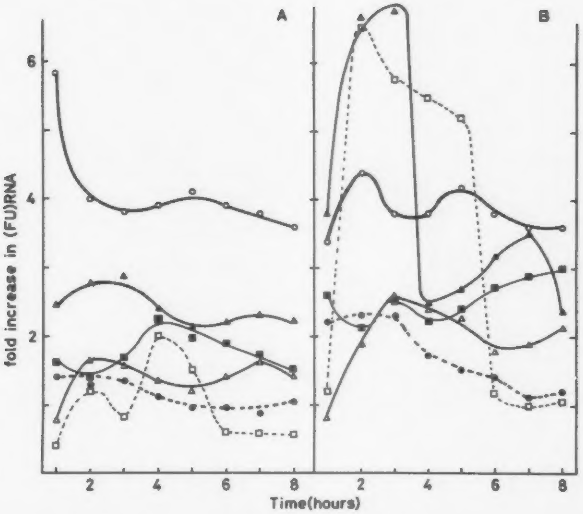

FIGURE 6.—Effect of dThd on the incorporation of ip administered FUra into RNA of the tumor and various organs. Tumor-bearing mice were treated by intratumoral (A) or ip (B) injection of dThd (100 mg/kg) with ip FUra (10 mg/kg) + $[^3H]$FUra (3 mCi/kg), as described in the fig 5 legend. At the indicated times, the tumor (○), bone marrow (●), intestine (△), kidneys (▲), lungs (□), and liver (■) were removed. The incorporation of radioactivity into RNA of these tissues was measured. Animals given intratumoral injection of saline and ip injection of FUra (10 mg/kg) + $[^3H]$FUra (3 mCi/kg) served as controls to calculate the relative increase in (FU)RNA. Each point represents mean of 3 values.

selective advantage in regard to incorporation of FUra into tumor cell RNA as compared to normal tissues.

Additionally, catabolism of FUra in the tumor and liver was studied (table 1). FUra in the tumor was degraded only by 0.7 nmol/g of tissue/minute, while FUra in the liver was degraded by 31.7 nmols. These results suggest that tumor cells have markedly lower levels of FUra-catabolizing enzymes than does liver. Thus, in this experimental system it seems likely that dThd's mode of increasing (FU)RNA may not involve inhibition of FUra degradation.

The potential for the therapeutic synergism between FUra and dThd was confirmed (fig 1). Ip FUra plus dThd (Group 7) and ip FUra plus intratumoral dThd (Group 6) were highly effective against the tumor. After 30 days from initiation of treatment, the tumor in both Groups 6 and 7 had grown only sixfold over the initial mean weight, in contrast to about 124-fold with saline treatment alone (Group 1). FUra alone (Groups 4 and 5) was moderately effective and suppressed the tumor growth by one-half after 30 days of treatment ($P < 0.05$). dThd alone (Groups 2 and 3) was therapeutically ineffective. Thus, the antitumor activity of FUra increased by 17-fold in the combined dThd and FUra ($P < 0.001$). These results were obtained with no increase in mortality. All 30 mice treated with dThd plus FUra survived.

TABLE 1.—Degradation of FUra in the tumor and liver

	FUra* degraded (nmols/g of tissue/min)			
Tissue	F-β-Ala	F-β-UPA	Others†	Total
Tumor	0.1	0.3	0.3	0.7
Liver	4.9	23.4	3.4	31.7

*80 nmols of FUra was used for assay.

†Phosphorylated products of FUra.

DISCUSSION

We found that intratumoral injection of dThd enhanced FUra incorporation into tumor RNA and antitumor activity of FUra administered systemically.

Two biochemical mechanisms have been linked to the cytotoxic effects of FUra. One mechanism involves conversion of FUra to FdUMP, which binds irreversibly to thymidylate synthetase, leading to the inhibition of DNA synthesis (1-3). The second mechanism is by the conversion of FUra to FUMP, which is incorporated into RNA, resulting in RNA dysfunction (16-18). Recently, it has been reported that the level of incorporation of FUra into DNA is also closely associated with cytotoxicity in mouse bone marrow cells (19). We favor the view that the RNA pathway plays a significant role in the antitumor activity of FUra, since increased incorporation of FUra into tumor RNA has been associated with enhancement of the antitumor activity of FUra in a number of in vitro (20–22) and in vivo studies (9–11,23,24). Of relevance to such observations, the synergic antitumor effects obtained with the combination of FUra and dThd have been confirmed in murine tumor systems (4–11). Noteworthy are the studies by Spiegelman et al (11) on two murine solid tumors (CDF_1 mammary carcinoma and $CD2F_1$ colon tumor 26), which were effectively treated by a combination chemotherapy regimen consisting of dThd and FUra. They found that combining drugs such as PALA, pyrazofurin, uridine, cytidine, and dThd with FUra altered incorporation into RNA and was associated with changes in antitumor activity. These results were obtained with systemic administration (ip injection) of FUra and dThd and/or other metabolic modulators. We observed in our murine tumor system that an intratumoral injection of dThd plus ip administration of FUra also resulted in increased formation of tumor (FU)RNA. Furthermore, the tumor used in our study possessed relatively low capacity for FUra degradation (table 1). These findings support the view that dThd may not only protect FUra from catabolic degradation in the liver or tumor but may also induce feedback repression of FUra anabolic pathways leading to deoxyderivatives, thereby encouraging the entry of FUra into RNA as reported by others (10,25).

In the study of the dose-response relationship of dThd to the incorporation of FUra into RNA (fig 4), the finding that treatment with doses > 100 mg/kg did not further increase formation of (FU)RNA may suggest an upper limit to such feedback repression and/or depression of RNA synthesis by the cytotoxicity of dThd itself (26–29).

In our experiments, intratumoral and ip injection of dThd increased (FU)RNA formation and the antitumor activity of FUra to essentially equal extents. However, the amount of (FU)RNA in several normal tissues, bone marrow, intestine, kidneys, and lungs was decreased by half in the case of intratumoral injection of dThd as compared to ip administration. This phenomenon is attributed to the lower dThd distribution in the normal tissues with intratumoral injection as compared to ip injection of dThd (fig 2), and the difference of dThd distribution in the normal tissues may result from variation in the rate of absorption of dThd into the systemic circulation and evacuation of the drug from the system. Thus, although differences in antitumor activity of ip versus intratumoral administration of dThd were not evident from this study, the potential for less severe side effects through the use of intratumoral dThd with systemic FUra is certainly a possibility in a clinical setting where drugs are administered at maximally tolerated doses.

Clinical trials on the combined infusion of FUra and dThd have been done to treat colorectal and breast carcinomas. The heretofore obtained responses have been low (13,30–32). It is possible that the use of intratumoral injection of dThd with systemic administration (iv) of FUra would decrease toxicity while leaving antitumor activity intact; this approach seems worth clinical evaluation in surface tumors, such as skin and breast cancer, and gastrointestinal cancers by means of endoscopical injection or intra-arterial administration into the hepatic artery for hepatoma or liver metastasis of colorectal cancer.

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Human Plasma Pharmacokinetics and Urinary Excretion of Thiotepa and Its Metabolites[1,2]

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Thiotepa has been used clinically for > 30 years but its pharmacokinetics remain poorly defined. We determined the plasma pharmacokinetics and urinary excretion of thiotepa and its metabolites in 21 patients with breast cancer who received 25 courses of iv bolus thiotepa (12 mg/m^2) as part of combination chemotherapy. Plasma samples were obtained before injection: at 5, 10, 15, 30, 45, 60, 90, and 120 minutes; and, when possible, 180 and 240 minutes after injection. In eight courses, urine was collected as 4-hour aliquots for 24 hours after therapy. All samples were analyzed for thiotepa and tepa by gas-liquid chromatography. Urinary alkylating activity was assessed spectrophotometrically after reaction with 4-(*p*-nitrobenzyl)-pyridine. Plasma concentrations of thiotepa declined in a biexponential fashion with an α-half-life of 7.7 ± 1.2 minutes and a β-half-life of 125 ± 21 minutes. Total-body clearance of thiotepa was 186 ± 20 ml/minute/m^2. The volume of the central compartment was calculated as 0.25 ± 0.04 L/kg, and the steady-state volume of distribution was calculated as 0.70 ± 0.11 L/kg. Tepa was detectable in plasma by 5 minutes after the injection of thiotepa. Tepa concentrations increased from 0.093 ± 0.068 to 0.127 ± 0.11 μg/ml over the 240-minute collection period. By 120 minutes, the concentration of tepa equaled that of thiotepa, and tepa persisted longer in the plasma than did thiotepa. During the first 24 hours after injection, urinary excretion of thiotepa, tepa, and alkylating activity accounted for 1.5%, 4.2%, and 23.5% of the administered dose, respectively. These results extend our laboratory's previous animal studies of thiotepa and argue for metabolism of thiotepa to tepa as a major mechanism of clearance of this compound. Further metabolism or breakdown of both compounds may explain the urinary excretion of alkylating materials other than parent compound and tepa. [Cancer Treat Rep 70:859-864, 1986]

Thiotepa (fig 1) is an alkylating agent, the clinical antitumor activity of which has been known for approximately 30 years (1). There has been a recent resurgence of interest in this compound. In current practice, thiotepa is administered systemically to treat metastatic carcinoma of the breast (2,3), intrathecally to treat carcinomatous meningitis (4,5), and intravesically to treat superficial carcinoma of the bladder (6,7). Reflecting the era of its development and initial clinical evaluation, pharmacological studies of thiotepa utilized methods such as radiolabeled drug (8-10), paper chromatography (9,11), fluorometry (12), and alkylating assays (13) that were relatively insensitive, cumbersome, and often nonspecific.

The paucity of pharmacological data concerning thiotepa raised the issue of whether the drug is currently used in a clinically optimal fashion. Recently, Grochow et al (14) developed a specific gas-liquid chromatographic (GLC) analysis of thiotepa, which we subsequently modified to allow simultaneous quantification of tepa (fig 1), the proposed major metabolite of thiotepa (15). We have now used this improved analytical methodology to define and characterize the plasma pharmacokinetics and urinary excretion of thiotepa and tepa in 21 patients who were receiving thiotepa as part of combination chemotherapy for breast cancer.

MATERIALS AND METHODS

Patient Population

All of the patients studied were receiving protocol combination chemotherapy for metastatic or recurrent carcinoma of the breast. To be eligible for treatment,

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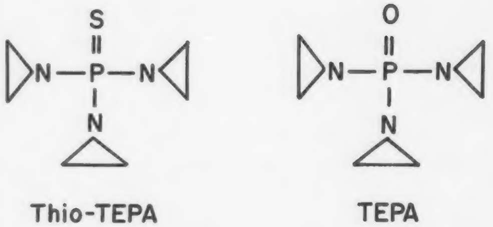

FIGURE 1.—Chemical structures of thiotepa and tepa.

each patient had to meet all of the following criteria: age ≥ 16 years; histologically documented metastatic carcinoma of the breast that was locally recurrent or unresectable; Karnofsky performance status ≥ 40%; life expectancy ≥ 2 months; at least one tumor mass measurable in two perpendicular diameters by either physical examination, roentgenography, or computerized axial tomographic scan; wbc count $\geq 3500/\mu l$; platelet count $\geq 100{,}000/\mu l$; hemoglobin ≥ 10 g/dl; serum urea nitrogen ≤ 25 mg/dl; serum creatinine ≤ 1.5 mg/dl; and serum bilirubin ≤ 1.5 mg/dl. For a bilirubin > 1.5 mg/dl, the dose was modified. Patients could have been either pre- or post-menopausal. Liver function tests (SGOT, SGPT, and alkaline phosphatase) were also performed as part of the pretreatment staging evaluation. Each patient was aware of the nature of her disease and gave written, informed consent for both therapy and pharmacokinetic studies. Therapy was administered in 28-day cycles and consisted of vinblastine (4.5 mg/m^2), doxorubicin (45 mg/m^2), and thiotepa (12 mg/m^2) iv on Day 1 and halotestin (10 mg orally three times per day) on Days 1-28.

Sample Acquisition

Heparinized blood samples were obtained before treatment; at 5, 10, 15, 30, 45, 60, 90, and 120 minutes; and, when possible, at 180 and 240 minutes after the injection of thiotepa. Samples were immediately centrifuged at $1000 \times g$ for 10 minutes. The resulting plasma supernatant was removed and stored at −20°C until analyzed. Eight courses of thiotepa were administered to hospitalized patients, and in these instances, urine was collected as 4-hour aliquots for 24 hours. Each aliquot was stored at 4°C until its collection was completed and then at −20°C until analyzed.

Sample Extraction and Analysis

Plasma or urine samples were thawed, and 0.1-ml aliquots were placed into 1.5-ml microcentrifuge tubes. The samples were extracted immediately with 0.4 ml of ethyl acetate containing diphenylamine (50 $\mu g/ml$) (Sigma Chemical Co, St. Louis, MO) as an internal standard. The samples were centrifuged at $12{,}000 \times g$ for 3 minutes in a Beckman Microfuge 12 (Beckman Instrument Co, Palo Alto, CA). One microliter of the ethyl acetate supernatant was injected into a Hewlett Packard 5840A gas chromatograph (GC) (Hewlett Packard, Palo Alto, CA) fitted with a 1.8-m × 2-mm (internal diameter) glass column containing 3% OV225 on 100/120 mesh Supelcoport (Supelco Inc, Bellefonte, PA). The oven and injection port were maintained at 180°C and 230°C, respectively. Nitrogen, at a flow rate of 30 ml/minute, was utilized as a carrier gas. The chromatograph was equipped with a nitrogen-phosphorus detector that was maintained at 230°C, with an air flow rate of 90 ml/minute, a hydrogen flow rate of 3.5 ml/minute, and a bead voltage of 16-20 V. Peaks were integrated with a Hewlett Packard 5840A GC terminal and thiotepa concentrations were calculated by comparison of their peak areas with that of the internal standard.

Because the previous method proved unsatisfactory for measurement of tepa, 22 courses were also analyzed by another procedure (15). Aliquots (0.5-ml) of plasma or urine were mixed with 0.1 ml of diphenhydramine internal standard (100 $\mu g/ml$) (Benadryl, Parke-Davis, Morris Plains, NJ). These mixtures were then loaded onto a SEP-PAK cartridge (Waters Associates, Milford, MA) which had been preconditioned by rinsing with 5 ml of high-performance liquid chromatography (HPLC)-grade methanol, followed by 5 ml of distilled, deionized water. After sample application, each SEP-PAK was rinsed with 5 ml of distilled deionized water and then was eluted with 2 ml of HPLC-grade methanol. The first 0.4 ml eluted from each SEP-PAK was discarded. The remainder of each methanol eluate was collected in 13 × 100-mm glass test tubes, dried under nitrogen, covered, and stored at −20°C until analysis when it was reconstituted in 25 μl of ethyl acetate. One microliter of the reconstituted sample was injected into the GLC system described above. Under these conditions, thiotepa, tepa, and the diphenhydramine internal standard had retention times of 4.15, 4.97, and 8.15 minutes, respectively (fig 2).

Alkylating activity in urine was assessed with 4-(*p*-nitrobenzyl)-pyridine by the colorimetric method of Friedman and Boger (16) and was quantified by comparison to simultaneously performed standard curves of thiotepa. When assessed by this colorimetric assay, alkylating activity was expressed in terms of thiotepa alkylating activity equivalents.

Pharmacokinetic Analysis

Computer modeling of the decline in plasma concentrations of thiotepa was accomplished with the MLAB program (17). Based on visual inspection, curves were fit to the sum of two exponentials by nonlinear regression with weighting by $(1/SD)^2$. Prior to fitting, initial estimates of A, B, α, and β were made by graphic analysis. Area under the curve (from zero to infinity) of plasma thiotepa concentrations versus time was calculated by the logarithmic trapezoidal rule with first-order extrapolation from the last data point. Total-body clearance was calculated as dose divided by the area under the curve of plasma thiotepa concentrations versus time. The volume of the central compartment and

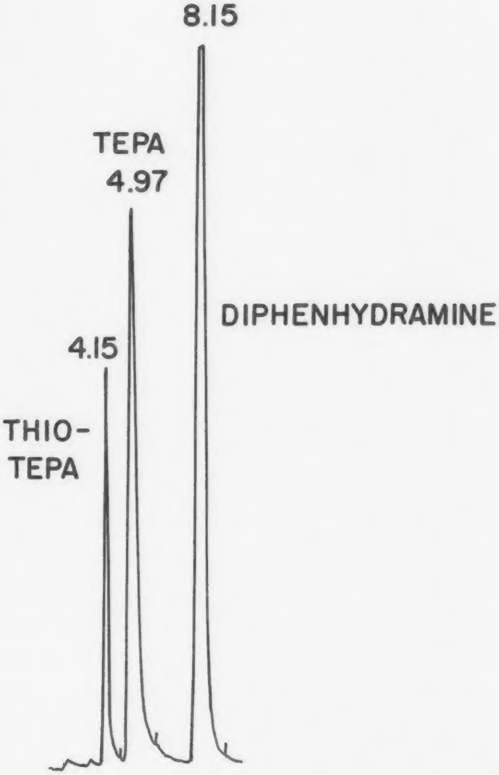

FIGURE 2.—Typical GC of a urine sample collected 4-8 hrs after an iv bolus injection of thiotepa at a dose of 12 mg/m^2. Urine was extracted with SEP-PAK cartridges and analyzed by GLC as described in the Materials and Methods section.

steady-state volume of distribution were calculated from a two-compartment analysis derived from the fitted parameters (18).

RESULTS

The 21 patients from whom samples were obtained had a median age of 57 years (range, 43–72) and a median Karnofsky performance status of 80% (range, 40%–100%) (table 1). One patient was studied on three separate courses and two patients were each studied on two separate courses. Each patient had disease involvement at multiple sites such as lung, bone, liver, or soft tissue. In compliance with protocol entry requirements, all patients had normal renal function. In contrast, a number of patients had elevations of one or more hepatic function tests. The three patients with serum bilirubin levels ≥ 1.5 mg/dl received reduced doses of thiotepa (one at 6 mg/m^2 and two at 9 mg/m^2).

Plasma concentrations of thiotepa decreased in a biexponential fashion (fig 3) with an α-half-life of 7.7 ± 1.2 minutes (mean ± SEM) and a β-half-life of 125 ± 21 minutes. The area under the curve of plasma concentrations versus time was 106 ± 30 μg/ml × minutes. From this value and the dosage of thiotepa administered, total-body clearance of thiotepa was calculated as 186 ± 20 ml/minute/m^2, the volume of the central compartment as 0.25 ± 0.04 L/kg, and the steady-state volume of distribution as 0.71 ± 0.11 L/kg.

Tepa was present at all analyzed times (fig 3), and its concentration remained approximately constant at 0.1 μg/ml. By 120 minutes after injection, the concentration of tepa in plasma equaled or exceeded that of thiotepa. Furthermore, tepa persisted in plasma longer than did thiotepa.

Thiotepa was excreted into the urine only during the first 8 hours and its excretion accounted for 1.5% of the total dose administered (fig 4). More than half of the

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Total	21
No. of courses: 25	
Median age in yrs (range): 57 (43–72)	
Median Karnofsky performance status (range): 80% (40%–100%)	
Sites of disease	
Soft tissue	20
Bone	15
Lung	5
Liver	6

Hepatic function tests	Median	Range	No. of patients with elevated values	Normal values
SGOT	43.5	(12–499)	8	(15–55 units/L)
SGPT	23.5	(6–139)	6	(4–32 units/L)
Bilirubin	0.4	(0.1–4.9)	4	(0.1–1.0 mg/dl)
Alkaline phosphatase	273	(109–705)	7	(95–300 units/L)

excretion of parent compound occurred during the first 4 hours and, as mentioned, was completed by 8 hours after injection. This pattern of urinary excretion reflected thiotepa plasma pharmacokinetics, since little parent compound remained in the plasma by 4 hours after administration. Tepa was excreted into the urine only during the first 12 hours after thiotepa administration, and its excretion represented 4.2% of the administered dose. The persistence of urinary excretion of tepa reflected the persistence of tepa in the plasma. In contrast, urinary excretion of compounds with alkylating activity continued over the entire 24-hour collection period and by 24 hours accounted for approximately 24% of the total administered dose of thiotepa. At no time could the total alkylating activity present in any urine sample be accounted for by the amount of thiotepa plus tepa present in that sample (fig 4).

DISCUSSION

Although the clinical utility of thiotepa was demonstrated > 30 years ago (1) and the drug represents one of the oldest chemotherapeutic agents still used in clinical oncology, there has been sustained interest in defining clinical roles for thiotepa (2-7). On the other hand, there has not been a corresponding effort to utilize modern analytical methods to delineate the pharmacokinetic behavior and metabolic pathways of thiotepa. The development of a sensitive and specific GLC assay for thiotepa by Grochow et al (14) and our alteration of that assay (15) to allow simultaneous measurement of tepa, thiotepa's proposed major metabolite (8-12), has allowed us to characterize the pharmacokinetics of both compounds in humans.

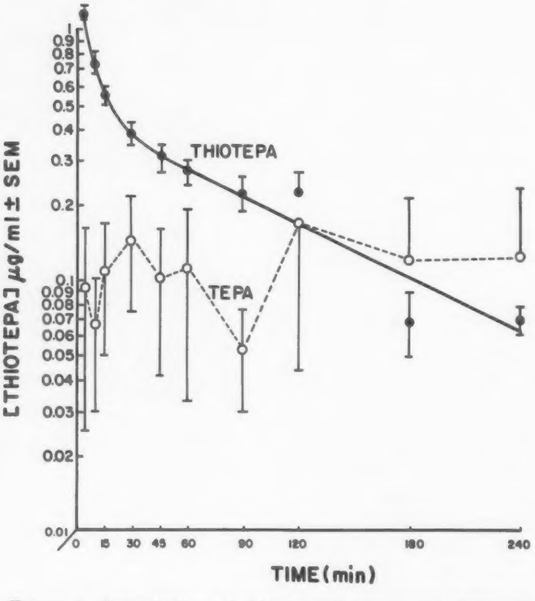

FIGURE 3.—Concentrations of thiotepa and tepa in the plasma of patients treated with an iv bolus injection of thiotepa at a dose of 12 mg/m^2. Solid line represents the computer-simulated line for thiotepa and points represent mean ± SEM of 25 courses (4 courses at 180 + 240 mins).

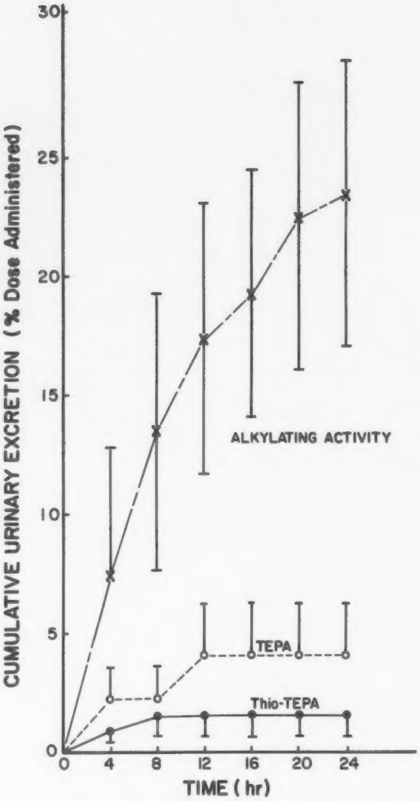

FIGURE 4.—Cumulative urinary excretion of thiotepa, tepa, and alkylating activity by patients treated with an iv bolus injection of thiotepa at a dose of 12 mg/m^2. Points represent mean ± SEM of 8 courses.

Our studies document the plasma pharmacokinetics of thiotepa as biexponential, with a terminal half-life of approximately 2 hours. In addition, the plasma behavior of thiotepa in humans is very compatible with our previously published data on the plasma pharmacokinetics and tissue distribution of thiotepa in mice (19). More specifically, in both species, thiotepa was distributed rapidly. The 0.71 ± 0.11 L/kg steady-state volume of distribution calculated for humans implies distribution throughout total-body water, a fact consistent with the widespread and even tissue distribution of thiotepa observed in mice (19). Furthermore, the 120-ml/minute/kg (360-ml/minute/m^2) total-body clearance of thiotepa calculated for mice proved a reasonable predictor of the 189-ml/minute/m^2 total-body clearance of thiotepa measured in humans. We recognize that our calculations of thiotepa's pharmacokinetic behavior are based on a sampling schedule which is less than ideal since only four patients were able to have plasma samples collected for > 120 minutes. Our confidence in our data is reinforced by subsequent studies in several patients who were treated with higher doses of iv thiotepa as a single agent, who had samples obtained for 24 hours after drug administration, and in whom thiotepa behaved as predicted by this current study (20). In addition, our patients all received thiotepa as part of a combination-chemotherapy regimen. Although this introduces the potential variable of drug-drug interaction, we feel the pharmacokinetics of thiotepa in our patients are representative because they agree with our earlier studies in mice (19) that were treated with thiotepa as a single agent, and because our subsequent studies have documented similar pharmacokinetic behavior of thiotepa in patients receiving the drug as a single agent (20).

Our current study documents the presence in both plasma and urine of tepa, the proposed major metabolite of thiotepa (8–12). Our measurement of these two compounds in each biological fluid has several implications. The presence and persistence of substantial concentrations of tepa in plasma raises the issue of its relative contribution to the antineoplastic activity of thiotepa, since tepa has well-documented in vivo and in vitro antiproliferative activity (22–25). In fact, the initial studies describing this subclass of alkylating agents showed tepa to be a more potent cytotoxic compound than thiotepa but also more difficult to work with due to its hygroscopic nature and tendency to polymerize (21–23). It may be that thiotepa serves, in part, as a prodrug, allowing for delivery of tepa in a more pharmaceutically suitable form.

The pattern of urinary excretion of tepa and thiotepa and the presence in urine of alkylating activity in forms other than tepa and thiotepa raises the question of what other potentially cytotoxic materials are generated from these two triethylenephosphoramide compounds. Oxidative desulfuration is known to occur and has been well-characterized with thio-organophosphorus insecticides such as parathion (25,26). We believe that this is the mechanism involved in the conversion of thiotepa to tepa and at present are trying to substantiate such a hypothesis. The facts that at no time do thiotepa and tepa account for all of the alkylating activity measured in urine and that excretion of alkylating activity continues long after excretion of thiotepa and tepa has ceased implies that the biotransformation of thiotepa does not stop with or is not limited to its conversion to tepa. We have already shown that incubation of thiotepa in urine at physiologic pH results in sequential conversion of the aziridine moieties of thiotepa to β-chloroethyl functions (27). The method of collection of urine in our current study was designed to minimize such as artifact. Similarly, we have shown that thiotepa is stable in plasma for prolonged periods of time. However, enzymatic conversion of aziridines to β-chloroethyl groups may occur in tissue and the resultant compounds may then be released into plasma and subsequently be excreted into the urine. Unfortunately, the analytical methodology employed in this study is unsuitable for analysis of such ring open compounds. In addition to compounds with open aziridine rings, aziridine itself may be liberated from either thiotepa or tepa (28). Such a process could be enzymatic, nonenzymatic, or possibly both. What role, if any, these potential metabolites play in the antitumor activity of thiotepa is at present a matter of conjecture. However, it is possible that further characterization of thiotepa's metabolic pathways and the associated metabolites will allow a more rational approach to chemotherapy with this agent.

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Phase I Study of Carboplatin (CBDCA) in Children With Cancer[1]

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A phase I study of carboplatin (CBDCA) was performed in 40 children with advanced cancer. A single course of CBDCA consisted of 4 weekly 1-hour infusions followed by a 2-week rest. The starting dose of 100 mg/m^2/week was 66% of the maximum tolerated dose in adults. Escalated dose levels given were: 125, 150, 175, and 210 mg/m^2. Myelosuppression was dose limiting, with thrombocytopenia more pronounced than leukopenia. There was no evidence of cumulative toxicity. The maximum tolerated dose for children with solid tumors was 210 mg/m^2/week × 4. Other side effects included transient nausea and vomiting at the higher dose levels and non-dose-related, reversible changes in creatinine clearance. One patient developed hives. No hepatic toxicity was seen. Among the 28 evaluable patients with solid tumors, one of ten with osteogenic sarcoma had complete disappearance of a lung nodule for 15+ months. Two of four patients with medulloblastoma had partial responses by clinical and computerized tomographic scan for 4 and 10 months. All three responders had received prior cisplatin therapy. CBDCA has major advantages over cisplatin in terms of reduced toxicity. Responses observed in patients previously treated with cisplatin are encouraging. The recommended phase II dose for children with solid tumors is 175 mg/m^2/week × 4 with a 2-week rest. [Cancer Treat Rep 70:865-869, 1986]

Cisplatin exerts substantial anticancer activity in a broad range of neoplasms in adults and children (1-7). Major dose-limiting toxic effects, such as severe nausea and vomiting, renal tubular damage, and high-frequency hearing loss, have led to the development of cisplatin analogs. Carboplatin (CBDCA) is a second-generation platinum analog which in preclinical trials has demonstrated equal antitumor effect with greater therapeutic index compared with the parent compound, cisplatin (8-9). Less nephrotoxicity and decreased emetogenic potential have been seen in animal studies with CBDCA (9). Its greater water solubility, diminished protein binding, and rapid renal clearance (compared with cisplatin) may account for the decreased renal toxicity seen in preclinical trials.

Several dose schedules have been studied in adult trials: 1-hour bolus and 24-hour continuous infusions every 28 days, bolus doses daily × 5, and weekly bolus doses × 4. All have shown myelosuppression as the dose-limiting toxic effect, with thrombocytopenia more pronounced than leukopenia (9-15).[3]

The purposes of this study were to determine the maximum tolerated dose (MTD) of CBDCA in children, to characterize the clinical toxicity, and to document any therapeutic effect in children with cancer who are resistant to conventional therapy.

PATIENTS AND METHODS

Forty patients were enrolled in this study (table 1). All patients had advanced cancer that was not responsive to conventional treatment. Prior therapy consisted of chemotherapy alone in 17 patients, 13 of whom had received cisplatin. Twenty-three patients had received radiation therapy in addition to chemotherapy; nine of these had received cisplatin. All patients had not received any therapy for 4 weeks or had recovered from the toxic effects of prior treatment.

Eligibility required creatinine clearance $\geq$ 70 ml/min/1.73 m^2; serum creatinine $\leq$ 1.5 mg/dl; bilirubin $\leq$ 2.0 mg/dl; and SGOT, gamma-glutamyl transpeptidase, and 5′-nucleotidase levels of no more than three times the upper limits of normal. Patients with solid tumors were required to have a wbc count $\geq$ 3000/mm^3 and a platelet count $\geq$ 100,000/mm^3. Patients with disease involving bone marrow had no hematological re-

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TABLE 1.—Patient characteristics

Characteristics	No. of patients
Entered	40
Male	28
Female	12
Median age in yrs (range): 14 (4–18)	
Prior therapy	
Chemotherapy alone	17*
Chemotherapy and radiation therapy	23†
Tumor types	
Bone sarcomas	13
Brain tumor	11
Soft tissue sarcoma	7
Neuroblastoma	1
Hodgkin's disease	1
Ovarian carcinoma	1
Nasopharyngeal carcinoma	1
Adrenal cortical carcinoma	1
Anaplastic carcinoma	1
Acute lymphoblastic leukemia	3

*13 had received prior cisplatin.
†9 had received prior cisplatin.

quirements, as assessment of drug effect in such cases would be inconclusive.

All patients had a comprehensive history and physical examination documenting subjective and objective evidence of disease prior to entry, with appropriate radiological and radionuclide studies. Baseline laboratory studies included a cbc with differential, platelet count, hepatic and renal function tests, urinalysis, and creatinine clearance. Audiometry was performed in selected patients. Follow-up studies included a weekly cbc with differential and biochemical screening. Repeat creatinine clearance and radiological and radionuclide studies were performed during the 2-week rest period. Written informed consent for participation in this study was obtained from the patients' guardians.

At least four patients were entered at each dose level, with additional patients at higher levels to better define the toxic effects. The initial dose was two-thirds of the MTD in adults, or 100 mg/m^2/week × 4 followed by escalations to 125, 150, 175, and 210 mg/m^2/week × 4. One patient with acute lymphoblastic leukemia received one course at 250 mg/m^2/week × 4. Dose escalation was permitted in the same patient after a course was completed if the disease was stable and toxic effects were acceptable. The MTD was defined as that dose which produced 2+ toxicity in 50%–70% with 3+ toxicity in 20%–30% of the patients. Life-threatening (4+) toxicity should be seen in $\leq 5\%$ of the patients who had received minimal prior therapy.

The criteria for response in patients with solid tumors was as follows: *complete response (CR)*—the disappearance of all measurable disease for a minimum of 1 month; *partial response (PR)*—a $\geq 50\%$ decrease of all measurable disease for a minimum of 1 month; *stable disease*—no objective change in any disease parameter; and *progressive disease*—an unequivocal increase in the size of existing disease or development of a new lesion.

Drug Preparation, Dose, and Schedule of Treatment

CBDCA was supplied in 20-ml amber vials containing 150 mg of the drug prepared as a white lyophilized powder with 150 mg of mannitol. The intact vials were stored under refrigeration (2°C–8°C). The drug was reconstituted at room temperature with 9.8 ml of sterile water for injection USP. Each milliliter of the resultant solution contained 15 mg of CBDCA and 15 mg of mannitol at pH 4.5–7.0. The drug was then mixed with 100–150 ml of 5% dextrose in water and given iv over 1 hour, without pre- or post-hydration. CBDCA was given weekly × 4 followed by a 2-week rest. This was considered to be a fully evaluable course.

RESULTS

Fifty courses of CBDCA were given to 31 patients. Nine patients failed to complete one course: six because of early deaths due to disease and three because they were taken off study with progressive disease after receiving two or three weekly doses. All patients were evaluable for acute toxicity. The toxicity grading was according to Miller et al (16), with minor modifications for pediatric patients.

Hematological toxicity in patients without bone marrow involvement is shown in table 2. Myelosuppression was the dose-limiting toxic effect. Leukopenia and thrombocytopenia appeared to be dose related, except at

TABLE 2.—Hematological toxicity of CBDCA as a function of dose level in patients with solid tumors*

Dose (mg/m^2/wk)	No. of patients	No. of courses	Median wbc count (× 10^3/mm^3)		Median days to —		Median platelet count (× 10^3/mm^3)			Median days to —	
			Initial	Nadir	Nadir	Recovery	Initial	Nadir	Range	Nadir	Recovery
100	2	6	4.8	3.7	29	35	232	90	42–182	42	56
125	4	7	3.9	3.5	22	32	235	169	21–198	36	42
150	5	6	4.2	3.0	15	19	256	125	112–301	41	50
175	10	12	5.2	2.8	32	44	246	93	14–179	32	42
210	7	8	6.8	2.3	39	47	354	41	11–152	36	45

*Excludes patients with marrow involvement.

the 100-mg/m^2 dose level (at which two heavily pretreated patients each receiving three courses were entered). One course at the 175-mg/m^2 dose level and three courses in three patients at the 210-mg/m^2 dose level resulted in a nadir absolute neutrophil count $<$ 500/mm^3. There were no episodes of fever or infection during any of the neutropenic periods. The nadir wbc and platelet counts occurred 1-2 weeks after the fourth weekly dose, with subsequent recovery within 2½ weeks. Aside from two heavily pretreated patients at the 100- and 125-mg/m^2 dose levels, platelet count nadirs $\leq 75 \times 10^3$ (grade 2 toxicity) were limited to the 175- and 210-mg/m^2 dose levels. Two patients died during the platelet count nadir period: one of progressive disease (carcinoma) and one of intratumoral (astrocytoma) brain hemorrhage. The latter patient died 1 week after her fourth weekly dose of 175 mg/m^2, with a platelet count of 20×10^3/mm.^{3} Autopsy demonstrated hemorrhage into the tumor bed. No changes in hemoglobin were observed in any patient. Among the five patients who received three or more consecutive courses of CBDCA, there was no evidence of cumulative hematological toxicity.

The major nonhematological side effects of CBDCA in all patients are shown in table 3. Transient nausea with vomiting requiring no hydration (grade 2 toxicity) was more common at the higher dose levels. The vomiting usually began 5–7 hours after the drug was infused, with resolution of symptoms within 18 hours.

No changes in serum creatinine were seen in any patient. A decrease in creatinine clearance of 40%–60% of a baseline clearance occurred in three patients at the 100-mg/m^2 dose level. Two of the patients showed transient decreases, with return to baseline values within 2 weeks, and the third died of progressive disease after two weekly doses. Two patients at the 210-mg/m^2 dose level displayed changes in creatinine clearance. One of these, who had metastatic adrenal carcinoma, showed a decrease from 79 to 5 ml/min/1.73 m^2, with a normal serum creatinine after receiving two weekly doses. This patient died 1 week later of progressive adrenal cortical carcinoma with massive compromise of the inferior vena cava (believed to explain the observed decrease in renal function) demonstrated at postmortem examination. The other patient showed a 50% decrease in creatinine clearance 3 weeks after the fourth weekly dose and died of progressive disease soon thereafter.

There were no alterations in liver function or electrolytes, including magnesium and calcium, attributable to CBDCA. One patient had a documented allergy (hives) to cisplatin and was premedicated with diphenhydramine and hydrocortisone prior to each dose of CBDCA. He completed four courses at the 100-mg/m^2 dose level without allergic reaction. Upon increase to the 175-mg/m^2 dose level for disease progression, he developed severe hives, resulting in discontinuance of CBDCA.

Patients receiving a minimum of one course of CBDCA were evaluable for therapeutic response (table 4). Of ten patients with osteogenic sarcoma, one 14½-year-old white male had pathologically proven complete disappearance of a 5-cm lung nodule. He was initially diagnosed with osteogenic sarcoma of the left distal femur. He received high-dose methotrexate and leucovorin rescue, cisplatin (120 mg/m^2/bolus), and doxorubicin, followed by amputation. Bilateral pulmonary metastases ensued. Treatment with high-dose methotrexate, bleomycin, cyclophosphamide, dactinomycin, vinblastine, and cisplatin (120 mg/m^2) provided no response. One month following bilateral thoracotomies, a nodule recurred which remained unchanged following one course of CBDCA (125 mg/m^2/week × 4). The nodule disappeared on x-ray 1 week after the patient completed a course of 150 mg/m^2/week × 4. An additional course of 150 mg/m^2/week × 4 was given, followed by a thoracotomy which showed fully necrotic osteogenic sarcoma. This patient is currently alive with no evidence of disease 21 months after start of CBDCA.

Clinical and computerized tomographic (CT) scan improvement was seen in two of four evaluable patients with recurrent medulloblastoma for 10 and 4 months, with a third patient demonstrating clinically stable disease for 9 months. A 9-year-old white male presented with recurrent medulloblastoma 3½ years after initial subtotal resection and radiation therapy. Treatment

TABLE 3.—Nonhematologic side effects of CBDCA in all patients

Dose (mg/m^2/wk)	No. of patients*	No. of courses				
			Nausea/vomiting		Renal function	
		Initiated	Evaluable	Grade 2	Evaluable	Grade 1
100	5	10	7	3	10	3
125	5	8	8	1	7	—
150	5	8	8	2	8	—
175	15	22	16	8	20	—
210	9	12	11	9†	12	2‡
250	1	1	1	—	1	—

*Patients counted once at level of entry on study.

†1 course with nausea only (grade 1).

‡1 patient with grade 4 toxicity. Patient died of progressive disease with massive compromise of inferior vena cava.

TABLE 4.—Therapeutic response in children with CBDCA

Diagnosis	No. of patients			Observed effects (mos)
	Entered	Evaluable	Disease progression	
Osteogenic sarcoma	12	10	6	1 CR (21+) lung nodule; 3 stable (6, 2, 1)
Medulloblastoma	5	4	1	2 PR (4, 10) clinical and CT*; 1 stable (9)
Embryonal rhabdomyosarcoma	4	2	2	
Carcinoma	4	4	3	1 stable (1¾)
Soft tissue sarcoma	3	3	3	
Astrocytoma	3	3	1	2 stable (1¼, 1)
Primitive neuroectodermal tumor	2	1	1	
Brain stem glioma	1	0	—	
Neuroblastoma	1	1	1	
Hodgkin's disease	1	0	—	
Ewing's sarcoma	1	0	—	
Acute lymphoblastic leukemia	3	3	3	

*Computerized tomography.

with cisplatin (120 mg/m^2/bolus × 4 courses) provided a PR with resolution of cord compression, but head CT scan and cauda equina abnormalities remained. He was then treated with melphalan and dianhydrogalactitol followed by autologous bone marrow transplant, with no change in disease. Having persistent disease on myelogram and CT scan, he was given CBDCA at 100 mg/m^2/week × 4. After completing one course of CBDCA, he achieved a CR by head CT scan for 10 months. Persistence of disease on myelogram accounted for his PR status. A 15-year-old white female developed recurrent medulloblastoma 5 years after subtotal tumor removal and radiation therapy. At that time she received additional radiation therapy and six courses of cisplatin (120 mg/m^2), with complete clinical and myelogram (cytology remained positive) and partial CT scan improvement for 1 year. Clinical and CT scan recurrence ensued 8 months off therapy. Four courses of CBDCA at 125 mg/m^2/week × 4 were given, with clinical improvement (within 2 weeks) and PR on CT scan. The PR was sustained for 4 months whereupon she developed progressive disease. An additional course was given at 175 mg/m^2 with no response.

Of the remaining 14 patients with solid tumors evaluable for response, all but three (stable disease) had progression of disease. Three patients with acute lymphoblastic leukemia each received one course at the 150-, 175-, and 250-mg/m^2 dose levels, respectively. Although the peripheral wbc count decreased by 18%-30% during all three courses, peripheral blasts and hypercellular blastic bone marrow remained.

DISCUSSION

CBDCA was evaluated in a phase I study to establish the MTD in children with cancer using a weekly × 4 bolus-dose schedule. The MTD for children with solid tumors was 210 mg/m^2/week × 4. At this dose, nausea and vomiting were observed in $> 80\%$ of the courses. Two patients had decreased creatinine clearance: one, grade 1 and the other, grade 4. The latter patient had a large tumor thrombus completely obstructing the inferior vena cava and died of progressive disease. The dose-limiting toxic effect was myelosuppression, with thrombocytopenia more pronounced than leukopenia. The myelosuppression was dose related and reversible in all evaluable cases. Among 40 patients entered on study, 28 of whom received 35 courses of CBDCA at the 175- or 210-mg/m^2 dose level, there was one probable drug-related death at the 175-mg/m^2 dose (hemorrhage into tumor during period of platelet count nadir). There was no evidence of cumulative toxicity. Nonhematological side effects consisted of mild transient vomiting and reversible changes in renal function. Allergy to platinum resulted in cessation of drug therapy in one patient.

Toxic effects revealed in this study were similar to those of other phase I studies using CBDCA on various schedules (9-15). The MTD, however, appears to be higher in children than the MTDs reported in adult studies. Using the same dosage schedule, Priego et al (14) reported an MTD of 150 mg/m^2/week × 4. Other adult studies using 24-hour infusion, bolus × 1, and bolus × 5 days every 28 days have suggested MTDs of 320-500 mg/m^2 (10-13). A pediatric phase I study using a similar bolus-dose every 28-day schedule reported 560 mg/m^2 as the MTD (15), which approximates the same amount of drug tolerated by children in this study.

Encouraging responses were seen in three patients: one with osteogenic sarcoma and two with medulloblastoma. Other reports have shown responses of varying degrees in ovarian, breast, head and neck, renal cell carcinoma, epidermoid carcinoma of the esophagus, and brain tumors (10,11,13,17-19).[4] The clinical spectrum of response to CBDCA is similar to that of response to cisplatin. A preliminary prospective randomized phase III study in ovarian carcinoma comparing CBDCA with cisplatin demonstrated equal response with markedly less toxicity with CBDCA (19).

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[4] Minutes of the Phase I Working Group Meeting, Investigational Drug Branch, National Cancer Institute, Bethesda, MD, Jan 19, 1984.

All three responders to CBDCA in this study had received prior cisplatin therapy with variable responses. The patient with pathological evidence of disappearance of a metastatic osteogenic sarcoma lung nodule had a poor response to cisplatin previously, following removal of prior lung nodules. This suggests possible lack of cross-resistance between CBDCA and cisplatin. Both patients with medulloblastoma demonstrated improvement following cisplatin therapy, for 4 and 12 months, with further cisplatin therapy withheld in one owing to compromised hearing loss.

Curt et al (10) reported minor responses in two patients with ovarian carcinoma who had previously demonstrated progressive abdominal disease while receiving cisplatin. Calvert et al (12) reported 24 patients with ovarian carcinoma who had received prior cisplatin therapy, ten of whom were resistant to cisplatin. One of these ten patients responded to CBDCA. Although the number of responders to CBDCA who demonstrated probable resistance to cisplatin is small, such evidence mandates future trials designed to evaluate the phenomenon of cross-resistance of cisplatin and CBDCA.

Based on this phase I study, a starting dose of 175 mg/m^2/week × 4 with a 2-week rest is recommended for children with solid tumors, with escalation to 210 mg/m^2/week, depending on bone marrow tolerance. Current phase II trials are ongoing to define therapeutic effectiveness in children with advanced bone sarcomas, soft tissue sarcomas, and brain tumors.

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Trial of High-Dose Cytarabine, Cyclophosphamide, Total-Body Irradiation, and Autologous Marrow Transplantation for Refractory Lymphoma[1]

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Twenty-one patients with advanced non-Hodgkin's lymphoma or Hodgkin's disease who had failed to be cured with standard therapy were the subjects of this clinical trial. The patients received cytarabine (3 g/m^2 at 12-hour intervals for six or eight doses), cyclophosphamide (90 mg/kg once), and total-body irradiation (one 900-cGy fraction or five 250-cGy fractions). Bone marrow was aspirated and cryopreserved before treatment and reinfused after the completion of radiotherapy. Eighteen patients (86%) had objective response and 12 (57%) achieved complete response. Three patients remain in continuous complete remission for 566+, 604+, and 1035+ days after marrow infusion. Six complete responders had tumor recurrence. One of these patients developed a localized lymphoma of another histology that was successfully treated with local radiotherapy and the patient is currently well 1004+ days after marrow infusion. Another relapsing patient responded to a brief course of salvage chemotherapy and is in remission at 1271+ days. Three of the complete responders died from infectious complications. This regimen was associated with significant toxicity. Six patients died from sepsis during the period of aplasia and three others died from interstitial pneumonia 42–105 days after marrow infusion. Although this regimen demonstrated a high level of antitumor activity, the value of adding high-dose cytarabine to the combination of cyclophosphamide and total-body irradiation remains unclear and would require a randomized clinical trial to demonstrate. [Cancer Treat Rep 70:871–875, 1986]

With presently available systemic therapies, many patients with advanced Hodgkin's disease and non-Hodgkin's lymphoma can be cured. However, some patients prove refractory to front-line chemotherapeutic agents, and others, despite initial complete remissions, eventually relapse. Presently available salvage chemotherapy regimens result in cure in a minority of patients with relapsed Hodgkin's disease and only rarely in patients with relapsed, aggressive non-Hodgkin's lymphomas. In the indolent non-Hodgkin's lymphomas, systemic chemotherapy does not appear to be curative.

Several previous reports have documented the activity of high-dose chemotherapy with or without total-body irradiation in patients with advanced lymphomas who have failed primary therapy (1–10). With presently available cryopreservation techniques, it is possible to salvage patients from otherwise lethal myelosuppression by infusion of autologous marrow. High-dose cytarabine is not used in any primary treatment regimen for lymphomas, but has recently been demonstrated to be active in patients with advanced disease (11–15). We performed a pilot study utilizing high-dose cytarabine, cyclophosphamide, total-body irradiation, and autologous marrow rescue in patients with Hodgkin's disease and non-Hodgkin's lymphomas who had failed to be cured with chemotherapy regimens administered in conventional doses.

PATIENTS AND METHODS

Criteria for entry in this study were: (*a*) the presence of a progressive non-Hodgkin's lymphoma or Hodgkin's disease that had failed to be cured with standard, systemic therapy, and (*b*) informed consent. Patients with Hodgkin's disease were considered to be incurable with conventional therapy after failing both mechlorethamine, vincristine, procarbazine, and prednisone (MOPP) and doxorubicin, bleomycin, vinblastine, and decarbazine (ABVD). Patients with aggressive non-Hodgkin's lymphomas were eligible for entry in the study after failing a front-line, doxorubicin-containing chemotherapy regimen. Patients with indolent non-Hodgkin's lymphomas were eligible only when they no longer responded to chemotherapeutic agents administered in

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standard doses. Twenty-three patients signed informed consent forms prior to entering the study. Two patients in very poor condition died before completing the preparative regimen. One patient with indolent non-Hodgkin's lymphoma died of a pulmonary embolus on Day −6 (before radiotherapy), and another patient with diffuse large cell lymphoma with an initial Karnofsky score of 40 died of sepsis and tumor progression after three doses of cytarabine. These patients were considered unevaluable and are not further analyzed. Twenty-one patients who received the planned preparative regimen and had their marrow reinfused form the basis of this report.

Patient characteristics are shown in table 1. The patients ranged in age from 15 to 52 years; there were 11 males and ten females. The diagnoses were diffuse large cell lymphoma in 17 patients (four after histologic transformation), Hodgkin's disease in two, diffuse undifferentiated lymphoma in one, and nodular poorly differentiated lymphocytic lymphoma in one. The initial Karnofsky scores varied from 40 to 90, with six patients having Karnofsky scores ≤ 70. Five patients had received previous radiotherapy. Ten of the patients had previously achieved complete remission with chemotherapeutic regimens administered in standard doses, but only two had achieved complete remission with their last chemotherapy regimen. Both of these patients received high-dose therapy as their initial treatment after relapse. All of the patients had active tumor at the time of entry in the study.

The treatment regimen consisted of high-dose cytarabine (3 g/m^2 administered at 12-hour intervals for six to eight doses), cyclophosphamide (90 mg/kg administered once), and total-body irradiation (administered in one 900-cGy fraction or five 250-cGy fractions). The first eight patients received cytarabine on Days −8, −7, −3, and −2; cyclophosphamide on Day −5; and total-body radiotherapy in one fraction on Day 0. It was then elected to use fractionated total-body radiotherapy at a dose of 250 cGy daily for 5 days. The next six patients received the cytarabine on Days −12, −11, −7, and −6 and the cyclophosphamide on Day −9. However, due to the long duration of therapy and, thus, the extension of the time of aplasia this was changed to the administration of the cytarabine on Days −10, −9, and −8 and the cyclophosphamide administered on Day −6. The fractionated total-body radiotherapy was administered on Day −4 through Day 0. Patients received steroid eye drops during the administration of cytarabine to avoid drug-related conjunctivitis. The patients were aggressively hydrated and had an indwelling Foley catheter placed for bladder irrigation during and 1 day follow-

TABLE 1.—Clinical parameters

Patient No.	Age/sex	Diagnosis*	Karnofsky score	Previous radiotherapy	Previous chemotherapy regimen†	Best previous chemotherapy result	Complete response with last treatment
1	32/M	DLC	80	No	CHOP, PVB	CR	No
2	34/F	NPDL	90	No	CVP, C, CHOP, ChlOP	CR	No
3	47/F	DLC	90	No	CAP-BOP	CR	Yes (relapsed)
4	40/M	DLC	90	No	CHOP	CR	Yes (relapsed)
5	25/M	DLC	90	Yes	CHOP, PVB	CR	No
6	16/M	HD	80	No	MOPP, ABVD	PR	No
7	26/M	DLC	80	No	CHOP	PR	No
8	24/F	DLC	80	Yes	CHOP	NR	No
9	29/M	DLC	70	No	CHOP	NR	No
10	42/M	NPDL→DLC	80	No	CVP, ChlOP, CAP-BOP	CR	No
11	47/F	DLC	40	Yes	CHOP, PVB	CR	No
12	16/M	DLC	90	No	CHOP, PVB	CR	No
13	39/M	HD→DLC	80	No	MOPP, CHOP	PR	No
14	15/F	DLC	80	No	CAP-BOP	PR	No
15	19/M	DLC	60	No	CHOP	PR	No
16	29/M	DU	80	No	CHOP, M-BACOD	PR	No
17	25/F	DLC	70	Yes	CHOP, COPP	CR	No
18	36/F	NPDL→DLC	70	No	CVP, CHOP, CP, Chl	CR	No
19	52/F	AILD→DLC	80	No	CHOP	NR	No
20	31/F	DLC	70	Yes	CHOP	PR	No
21	22/M	HD	80	No	MOPP, ABVD	PR	No

*DLC = diffuse large cell lymphoma; NPDL = nodular poorly differentiated lymphocytic lymphoma; HD = Hodgkin's disease; DU = diffuse undifferentiated lymphoma; AILD = angioimmunoblastic lymphadenopathy; and arrows = histologic transformation before entering this study.

†CHOP = cyclophosphamide, doxorubicin, vincristine, and prednisone; PVB = cisplatin, vinblastine, and bleomycin; CVP = cyclophosphamide, vincristine, and prednisone; C = cyclophosphamide; ChlOP = chlorambucil, vincristine, and prednisone; CAP-BOP = cyclophosphamide, doxorubicin, procarbazine, bleomycin, vincristine, and prednisone; M-BACOD = methotrexate, bleomycin, doxorubicin, cyclophosphamide, vincristine, and dexamethazine; COPP = cyclophosphamide, vincristine, procarbazine, and prednisone; CP = cyclophosphamide and prednisone; and Chl = chlorambucil.

ing the cyclophosphamide administration. Patients were cared for in reverse isolation but not in laminar air flow rooms.

Marrow was aspirated from 20 to 21 patients at a time of active disease. One patient had his marrow harvested in complete remission 14 months before treatment. The marrow was removed from the posterior iliac crest in the operating room under general anesthesia. The marrow was initially mixed with heparin and tissue culture medium. When 2×10^8 nucleated marrow cells/kg of patient weight were obtained, the specimen was filtered to produce a single-cell suspension. The volume was then reduced by removal of plasma and some rbcs. This was replaced with tissue culture medium containing DMSO to yield a final concentration of 10%. The cells were frozen in a controlled-temperature freezer at 1°C/minute and stored in liquid nitrogen until use. After administration of the last dose of radiotherapy, the cells were taken directly from the storage freezer to the patient's room, thawed quickly in a 40°C water bath, and immediately infused iv. Patients were aggressively hydrated before and during the marrow infusion. Because erythrocytes were not removed from the marrow before freezing, patients regularly developed hemoglobinuria following the marrow infusion.

Complete response is defined as the absence of tumor after the completion of therapy. Documentation was either by clinical (ie, physical examination, laboratory evaluation, and imaging studies) or biopsy re-evaluation of sites known previously to contain disease or at autopsy. All previously abnormal tests were repeated for restaging. Partial response was defined as at least a 50% shrinkage of all known disease. All other patients were considered nonresponders. Survival times and response durations were calculated from the day of marrow infusion. Informed consent for participation in this study as approved by the Institutional Review Board at each institution was obtained from each patient.

RESULTS

The outcome of treatment is shown in table 2. Eighteen of the patients (86%) had objective tumor response and 12 (57%) had complete response. Three patients (14%) demonstrated no tumor shrinkage. Three complete responders remain alive in continuing complete remission for 566+, 604+, and 1035+ days. Five patients who had complete response had subsequent relapse of the same tumor from 59 to 238 days after marrow infusion. One of these patients with biopsy-proven recurrence had complete response to three cycles of salvage chemotherapy using cisplatin, vinblastine, and bleomycin and remains well and disease-free at 1271+ days after marrow infusion. One patient with nodular poorly differentiated lymphocytic non-Hodgkin's lymphoma had complete response but developed a localized large cell lymphoma in a right supraclavicular lymph

TABLE 2.—Response to high-dose therapy*

	Treatment doses			Treatment Outcome			
Patient No.	Cytarabine (g/m^2)	Cyclophosphamide (mg/kg)	Total-body irradiation (cGy)	Tumor response	Complete response duration (days)	Survival (days)	Cause of death
1	3 × 8	90 × 1	250 × 5	CR	1035+	1035+	—
2	3 × 8	90 × 1	250 × 5	CR	370	1004+	—
3	3 × 6	90 × 1	250 × 5	CR	604+	604+	—
4	3 × 6	90 × 1	250 × 5	CR	566+	566+	—
5	3 × 8	90 × 1	250 × 5	CR	238	523	Lymphoma
6	3 × 8	90 × 1	900 × 1	CR	120	754	Lymphoma
7	3 × 8	90 × 1	900 × 1	CR	90	227	Lymphoma
8	3 × 8	90 × 1	900 × 1	CR	79	105	IP
9	3 × 8	90 × 1	900 × 1	CR	59	1271+	—
10	3 × 6	90 × 1	250 × 5	CR	20+	20	ARF + sepsis
11	3 × 8	90 × 1	250 × 5	CR	9+	9	ARF + sepsis
12	3 × 8	90 × 1	250 × 5	CR	6+	6	Sepsis
13	3 × 8	90 × 1	900 × 1	PR	—	232	Lymphoma
14	3 × 6	90 × 1	250 × 5	PR	—	153	Lymphoma
15	3 × 6	90 × 1	250 × 5	PR	—	72	Lymphoma
16	3 × 6	90 × 1	250 × 5	PR	—	42	IP
17	3 × 6	90 × 1	250 × 5	PR	—	10	Sepsis
18	3 × 6	90 × 1	250 × 5	PR	—	14	Cerebral hemorrhage
19	3 × 8	90 × 1	900 × 1	NR	—	56	IP
20	3 × 8	90 × 1	900 × 1	NR	—	14	Sepsis
21	3 × 8	90 × 1	900 × 1	NR	—	8	Sepsis

*CR = complete response; PR = partial response; NR = no response; ARF = acute renal failure; and IP = interstitial pneumonia.

node 370 days after marrow infusion. This was successfully treated with local radiotherapy and the patient remains well at 1004+ days. Three of the complete responders died from infections and had no tumor found at autopsy. Although the chance for achieving complete response was similar with total-body irradiation administered in one 900-cGy fraction (four responses among eight patients, 50%) and with 1250-cGy fractionated total-body irradiation (eight responses among 13 patients, 52%), the responses were not equally durable. All four patients who received 900 cGy as single-fraction total-body irradiation have relapsed in contrast to two of five patients who received fractionated total-body radiotherapy and had comparable follow-up.

The myelosuppression associated with this regimen was severe, with all patients reaching a 0 granulocyte count and all becoming dependent on platelet and rbc transfusions. The patients had from 1.3×10^8/kg to 3.3×10^8/kg nucleated marrow cells infused. The median time from marrow infusion to recovery of a granulocyte count of 0.5×10^9/L was 22 days, with a range of 8–34 days. A total wbc count of 1.0×10^9/L was achieved in a median of 23 days, with a range of 13–45 days. A stable platelet count of $\geq 20 \times 10^9$/L independent of transfusion was achieved in a median of 28 days. Three patients who recovered other counts died without having platelet count recovery and one patient did not reach a platelet count of 20×10^9/L without transfusion until $>$ 100 days. All patients had a paucity of megakaryocytes in the marrow and none had any evidence to support an autoimmune thrombocytopenia. A reticulocyte count of 50×10^9/L was reached in a median of 34 days. One patient who recovered granulocyte production died without achieving a reticulocyte count at this level. Granulocyte transfusions were not used for any of the patients in this series. The patients required a mean of 14 units of packed rbcs (range, 3–44 units) and 104 units of platelet concentrates (range, 12–210 units).

This regimen was associated with significant toxicity. Of the 16 patients who have died, ten died from treatment-related complications and six from progressive lymphoma. All of the patients became febrile and required antibiotics during the period of aplasia, and six died from septic complications. The major infecting organisms were *Pseudomonas aeruginosa* in one patient, *Streptococcus viridans* in one patient, *Histoplasma capsulatum* in one patient, and *Candida* species in three patients. Two of the patients who died from candidal septicemia had developed acute renal failure coincident with the infusion of the marrow. Three patients developed idiopathic interstitial pneumonia and died 42, 56, and 105 days after the marrow infusion. One patient who became refractory to platelet transfusions died from a cerebral hemorrhage on Day +14. Nausea, vomiting, and diarrhea occurred to a mild to moderate degree in all patients. Conjunctivitis and central nervous system toxicity due to cytarabine were not observed.

DISCUSSION

High-dose cytarabine has been utilized as a single agent in patients with relapsed non-Hodgkin's lymphoma (11-15). The drug demonstrated considerable, albeit shortlived, antitumor activity and was associated with relatively mild, extramedullary toxicity. High doses of cytarabine have also been included in a preparative regimen for allogeneic bone marrow transplantation for acute leukemia (16). We incorporated high-dose cytarabine into a very intensive treatment regimen for use in patients with refractory non-Hodgkin's lymphoma and Hodgkin's disease in combination with high-dose cyclophosphamide, total-body irradiation, and autologous marrow rescue. This combination had a very high level of antitumor activity, even in these patients with advanced and generally refractory disease. Nine of 13 patients with de novo diffuse large cell lymphoma (69%) achieved complete response and three (29%) are in continuous remission for from 566+ to 1035+ days.

The combination of high-dose cytarabine, cyclophosphamide, and total-body radiotherapy was associated with a high treatment-related toxicity and death rate. Ten of the 21 patients (48%) died from treatment-related complications. Six patients (29%) died from sepsis during the period of aplasia. Both the total treatment-related mortality rate and the frequency of septic deaths are higher than we have observed for allogeneic transplantation for leukemia using this regimen and for autologous marrow transplantation using different regimens for other diseases. It has been previously suggested that patients with lymphoma who enter treatment with a defect in cellular immunity and then undergo the severe myelosuppression associated with the high-dose treatment regimen might be at an unusually high risk for fatal infections (17). Because of this propensity to infection, it is probably important to complete the preparative regimen as quickly as possible and not extend the period of aplasia. The longest sequence of therapy that we used (ie, total of 12 days) left patients with no peripheral granulocytes by Day −4. In light of this experience, we recommend using a preparative regimen that can be completed in 1 week so patients do not have severe granulocytopenia before the marrow is reinfused.

The possibility of marrow contamination by malignant cells has been a concern with autologous marrow rescue for hematopoietic malignancies. All of the patients in this series had histologically negative marrows at the time of the marrow collection and storage. Only the patient with nodular poorly differentiated lymphocytic lymphoma and two with diffuse large cell lymphoma had previously had documented marrow involvement. In these patients the marrow was cleared with chemotherapy at conventional doses before marrow storage. Despite this, the patient in our series with diffuse undifferentiated lymphoma had tumor recurrence in the marrow coincident with the reappearance of hematopoiesis. This patient raises the question of inapparent marrow contamination and "transplantation" of the tumor. In all the other patients in this series in whom the lymphoma reappeared, it was at sites of previously known, bulky disease.

In summary, high-dose therapy with cytarabine, cyclophosphamide, and total-body irradiation had a high response rate in patients with refractory lymphoma and yielded long-term disease-free survival in patients with diffuse large cell lymphoma who failed to be cured with standard doses of therapy. However, it is not clear that the addition of high-dose cytarabine to the combination of cyclophosphamide and total-body irradiation significantly increased the response rate. The great variation in clinically significant variables such as timing of the therapy (ie, remission vs relapse), tumor bulk, tumor responsiveness to previous therapy, patients' general health, and patient age makes comparison of results from different series difficult. It is possible that differences in patient characteristics are a bigger variable when comparing results than differences in the currently available treatment regimens. This question would require a randomized controlled study to answer. However, high-dose therapy and autologous marrow rescue can be curative in patients with lymphomas that fail standard chemotherapy regimens. Since salvage regimens at conventional doses are rarely curative, this treatment approach should be considered when primary chemotherapy regimens fail.

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Pharmacokinetics and Immediate Effects of High-Dose Carmustine in Man[1,2]

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The pharmacokinetics and acute toxicity of carmustine (BCNU) have been studied in ten patients receiving high-dose combination chemotherapy with cyclophosphamide, cisplatin, and BCNU as treatment for advanced neoplasms. Patients received from 300 to 750 BCNU mg/m^2 of body surface area as a 2-hour infusion. The immediate effects of this schedule of BCNU included tachycardia, hypotension, flushing, confusion, nausea, and vomiting. Hypotension was a prominent feature of high-dose BCNU administration. The pharmacokinetics of high-dose BCNU were studied via serial blood samples obtained during and following BCNU infusion. Concentrations of BCNU in total plasma and ultrafiltrable (bioavailable) plasma were determined by high-pressure liquid chromatography with UV detection. Average pharmacokinetic parameters for bioavailable plasma BCNU, calculated on the basis of a one-compartment model, include an elimination constant of 0.031 min^{-1} and a volume of distribution of 5.1 L/kg. Average clearance of total plasma BCNU is 77.6 ml/kg/min. When corrected to a constant dose of 1 g/m^2, the average peak concentration at the end of the infusion was 7.8 μM and the area under the curve was 538 μM·min. Plasma BCNU was largely (77%) protein bound. The distribution, clearance, and protein binding of high-dose BCNU were similar to those reported for standard-dose BCNU. [Cancer Treat Rep 70:877-880, 1986]

Carmustine (BCNU) is an antineoplastic drug widely used for the treatment of human solid tumors and lymphomas (1,2). BCNU is administered as a single agent at doses of 50-210 mg/m^2 and in combination chemotherapy regimens at similar or lower doses. BCNU is also a component of some high-dose chemotherapy regimens with autologous bone marrow reinfusion, either as a single agent (3-5) or in combinations (6,7). BCNU at high doses has increased therapeutic efficacy (8) but also produces novel toxic effects including hepatic dysfunction, pneumonitis, and dementia (3,5,9).

The pharmacokinetics of BCNU in man have been less extensively studied than the pharmacokinetics of some other antineoplastic agents. In part, this is due to the difficulties of quantitating the drug and also to its instability in solution. The most extensive pharmacokinetic study of BCNU in humans was published by Levin et al (10). Twenty patients receiving bolus injections of 45-170 mg/m^2 were studied via selected ion monitoring using a chemical ionization direct-probe mass spectroscopy technique. Plasma concentration-time data were analyzed with a two-compartment open model. The volume of distribution at steady state was 3.25 L/kg and the plasma clearance was 56 ml/min/kg. The rate at which BCNU transfers to the outside from the central compartment (0.032 min^{-1}) was close to the rate at which BCNU decomposed in vitro in patient serum (0.044 min^{-1}). Substantial patient-to-patient variation in the plasma clearance of BCNU was observed. Levin et al (10) speculated that such variation might be dependent on the patient's percent of body fat and serum lipid content.

The goal of this study was to determine the pharmacokinetics of high-dose BCNU in a regimen using a combination of alkylating agents. Both the time course of BCNU concentrations in plasma and the short-term cardiovascular effects are described in this report.

MATERIALS AND METHODS

Patient selection and treatment.—Blood samples for this study were obtained from patients receiving BCNU

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TABLE 1.—Bioavailable BCNU in plasma

Patient No.	Drug (mg/m^2)			AUC* ($mM \cdot min$)	C_{max} (μM)	$t_{1/2}$ (min)	V_{DIS} (L/kg)	Clearance (free) (ml/min/kg)	Bioavailable drug (% of total)	Clearance (total plasma) (ml/min/kg)
	BCNU	Cyclophosphamide	Cisplatin							
1	300	3000	120	0.154	4.8	—	—	267	37	99
2	450	3750	150	0.114	1.7	—	—	425	15	64
3	600	5625	165	0.139	3.0	10	2.7	491	19	93
4	600	5625	165	0.196	3.7	19	5.6	414	32	132
5	600	5625	165	0.323	4.4	17	3.6	194	20	39
6	600	7500	180	0.332	2.6	29	10.2	225	26	58
7	600	5625	165	0.410	3.7	24	7.6	182	20	36
8	600	5625	165	0.578	5.0	24	3.7	111	21	23
9	600	5625	165	0.887	10.8	33	2.5	83	18	15
10	750	7500	180	0.102	1.8	—	—	868	25	217
Average	570	5550	162	0.322†	4.7†	22.2	5.1	326	23.3	77.6

*AUC = area under the curve.

†Values adjusted to a standard dose of 600 mg/m^2 prior to calculation of averages.

as part of a phase I study of combination alkylating agents administered with autologous bone marrow reinfusion (7,9). The three-drug treatment was administered on the following schedule: cyclophosphamide over 2 hours on Days 1, 2, and 3; cisplatin as a continuous iv infusion on Days 1-3; and BCNU at 5 $mg/m^2/min$ on Day 4. Solutions of BCNU for iv infusion were prepared exactly as recommended by the manufacturer in the package insert. The doses of BCNU and other drugs administered for the ten patients studied are shown in table 1. Informed consent was obtained from all patients, and all requirements for institutional review were fulfilled.

Sample collection, storage, and analysis.—All patients had indwelling double-lumen venous catheters (Hickman) placed into the superior vena cava at the time of bone marrow harvest. Blood samples (5 ml) were collected through this catheter into prechilled tubes containing EDTA, and plasma was separated by centrifugation. For determination of bioavailable BCNU, plasma was centrifuged through Amicon ultrafilters ($M_r < 20{,}000$). Samples (1 ml) were deproteinized by the addition of 2 ml of methanol and centrifugation at 10,000 × *g* for 10 mins. The supernatant was stored at −20°C until analysis, which was performed within 10 days. Samples were analyzed for BCNU concentrations by a modification of the method of Krull et al (11). To 1 ml of the sample was added 1 µl of a 5 mM solution of *p*-chloroaniline as internal standard. The mixture (10-60 µl) was injected onto a high-pressure liquid chromatography system that consisted of a C18 reverse-phase column (Waters) and a mobile phase of 1 mM ammonium acetate (pH 3.5): ethanol (90:10, vol/vol). The flow rate was 1.0 ml/min. A variable wavelength UV detector was set to 237 nm. Under these conditions BCNU eluted at 8 mins and internal standard (*p*-chloroaniline) at 11 mins. A standard curve for the ratio of peak height of BNCU to internal standard peak height was prepared using plasma samples spiked with 0.2-50 µM BCNU.

Calculation of pharmacokinetic parameters.—The concentration and time data obtained from each patient studied were used to calculate the pharmacokinetic parameters for BCNU. The concentration-time curve for plasma BCNU showed a simple exponential decline following the end of infusion. Therefore, we have chosen to analyze the data on the basis of a one-compartment model with iv infusion and first-order elimination (12). The first-order elimination rate, k_e, was calculated according to the least-squares best-fit of the relationship

$$\ln C(t) = \ln C_{max} - k_e t$$

for concentration-time data following the end of infusion, where C_{max} is drug concentration at the end of infusion. The $t_{1/2}$ of elimination is related to the elimination rate constant

$$t_{1/2} = 0.693/k_e.$$

The volume of distribution (V_D) was calculated according to the relationship

$$V_D = \frac{k_o}{C_{max}\, k_e}(1 - e^{-k_e t_o}),$$

where k_o is the rate of infusion and t_o is time at the end of infusion. The area under the curve (AUC) was calculated by the linear trapezoidal method (12). The clearance (Cl) was calculated according to the relationship

$$Cl = dose/AUC.$$

RESULTS

The immediate effects of an infusion of 600 mg/m^2 of BCNU on heart rate and blood pressure are shown in figure 1. The magnitude of the effect of this dose on blood pressure and heart rate for a group of 15 consecutive patients varied from patient to patient, but the time course of these effects was consistently that shown. The average change in heart rate was +25/min and the average change in blood pressure was −31 mm Hg systolic and −26 mm Hg diastolic. Tachycardia and hypotension, associated with intense flushing of the skin of the face and upper chest, were usually maximal before

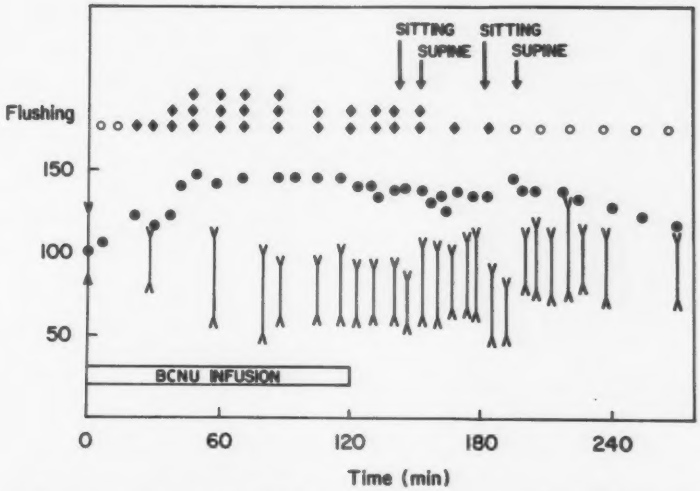

FIGURE 1.—Immediate cardiovascular effects of BCNU infusion. Clinical observations on patient 9 (table 1) receiving 600 mg/m^2 of BCNU by iv infusion from 0 to 120 mins. Pulse (●), systolic (Y), and (⅄) diastolic blood pressure are shown. Flushing was subjectively graded as absent (○), present (◆), moderate (◆◆) or severe (◆◆◆).

the end of infusion and persisted beyond the end of infusion, often for several hours. Orthostasis continued up to 3 hours beyond the end of infusion (fig 1). Blood pressures of 70/20, 74/30, 80/50, and 74/30 developed in four patients, requiring slowing or discontinuation of the BCNU infusion. Iv fluids were administered, and the infusion was completed when hypotension resolved.

No serious complications resulted from the acute cardiovascular effects of BCNU. However, considerable patient discomfort and anxiety were associated with the subjective "burning" associated with flushing, and several patients became confused while hypotensive.

The pharmacokinetics of high-dose BCNU were studied in ten patients receiving this regimen. Seven of ten patients received 600 mg/m^2 of BCNU. A typical time course of ultrafiltrable (bioavailable) plasma BCNU concentrations is shown in figure 2. Measurable levels of BCNU were usually present by 30 mins. Peak levels of 1.7-10.8 μM were reached at the end of infusion. For seven patients, sufficient samples were available to calculate $t_{1/2}$ and V_D according to a one-compartment first-order elimination model (table 1). Following termination of the infusion, BCNU levels declined, with an average $t_{1/2}$ of 22.2 mins (range, 10–33). High-dose BCNU has a V_D of free drug of 5.1 L/kg of body weight. The large apparent V_D is due in part to the high fraction of BCNU bound to plasma proteins (77%). Exposure to bioavailable drug ranged from 82 to 887 μM·min when adjusted to a 600-mg/m^2 dose.

The average clearance of bioavailable BCNU was 326 ml/min/kg of body weight (range, 83–868). To allow comparison with the study of Levin and co-workers, the clearance of total plasma BCNU in this study has been calculated by correcting for the percentage of drug bound to macromolecules. The average clearance of total plasma BCNU in this study was 77.6 ml/min/kg.

DISCUSSION

High-dose chemotherapy with autologous marrow reinfusion is being studied in the therapy of leukemias, lymphomas, and solid tumors (8). We have studied the events during and immediately following high-dose BCNU infusions. The cardiovascular effects of BCNU appear to be dose related: they have been reported following high- but not standard-dose regimens. However, there is no exact correlation between plasma drug concentration and hypotension or tachycardia. Both side effects persist beyond the period when BCNU levels are detectable. Furthermore, the extent of hypotension did not correlate with drug concentrations. Hypotension was a prominent feature of this schedule of high-dose therapy and has been noted in a previous study of high-dose BCNU (5). While no life-threatening complications of hypotension occurred in this study, Takvorian et al (5) reported a patient in whom hypotension and a myocardial infarction developed during high-dose BCNU therapy. Side effects during high-dose BCNU treatment were moderately severe with flushing, nausea, vomiting, orthostasis, and sometimes confusion.

It is not possible in this study to determine whether all the cardiovascular effects observed were due solely to BCNU administration. It is possible that the prior administration of cyclophosphamide and cisplatin had sensitized these patients to the cardiovascular effects of BCNU. The BCNU vehicle contained ethanol and it is also possible that the ethanol may have contributed to BCNU's cardiovascular effects. However, levels of

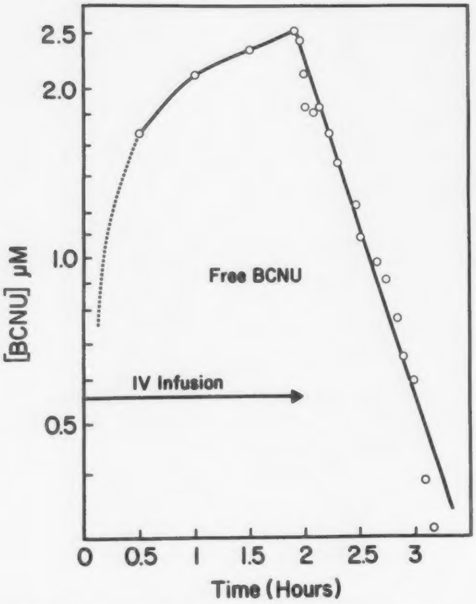

FIGURE 2.—Time course of concentration of plasma ultrafiltrable BCNU. At the indicated times, blood samples were obtained from Patient 6, who received a 2-hr infusion of 600 mg/m^2 of BCNU. Concentrations of plasma ultrafiltrable BCNU were determined as described in the Methods section.

plasma ethanol (< 50 mg/dl) were determined for two patients at the end of BCNU infusion and it is unlikely that these levels of ethanol alone were sufficient to cause hypotension. In a previous study (5), dehydration was felt to contribute to the BCNU-induced hypotension. However, patients in this study were well hydrated and had normal central venous pressures during BCNU infusion. Rather, the constellation of flushing and tachycardia suggest that hypotension may result from profound peripheral vasodilatation. It seems likely that BCNU alone, or perhaps BCNU and ethanol in synergy, acts directly to cause peripheral vasodilatation and that the tachycardia is compensatory. The mechanism by which BCNU produces vasodilatation is unknown.

Partly as an attempt to reduce these immediately toxic effects, we have altered the schedule of the administration of BCNU to deliver the same 600 mg/m^2 in eight divided doses given every 12 hours. No hypotension has been observed in the first five patients treated on this schedule. The effect of this alteration of schedule on therapeutic efficacy is not yet known.

The average pharmacokinetic parameters for this group of ten patients receiving high-dose BCNU are similar to the average values obtained by Levin et al (10) for a group of 20 patients receiving a fourfold- to tenfold-lower dosage. The two studies differ in several aspects. Levin's patients received bolus injections of BCNU, and he analyzed the data on the basis of a two-compartment model. Patients in this study received 2-hour infusions. No evidence for a second compartment was seen in the concentration-time data obtained from this study and the data were analyzed using a one-compartment model. However, the noncompartmental parameter of clearance is independent of the method of administration and the kinetic model selected. Plasma clearance in the low-dose BCNU study averaged 56 ml/min/kg, while clearance in this high-dose study averaged 77 ml/min/kg. These values are not significantly different. Both studies also observed a large (greater than tenfold) patient-to-patient variation in the clearance of BCNU. The concomitant wide range in AUC indicates substantial patient-to-patient variation in drug exposure, perhaps accounting for the range in severity of the myelosuppressive and nonmyelosuppressive toxic effects of BCNU (5).

In this small group of ten patients five developed hepatotoxicity, either noninfectious hepatitis (one) or veno-occlusive disease of the liver (four). Patients with high AUC for BCNU tended to have a higher incidence of subsequent hepatotoxicity ($P < 0.17$ by logistic regression). Further study of the relationship between the pharmacokinetics of BCNU and therapeutic and toxic outcomes is warranted.

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Toxicity of Oral *N*-Methylformamide in Three Phase II Trials: A Report From the National Cancer Institute of Canada Clinical Trials Group[1]

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Three National Cancer Institute of Canada phase II studies of *N*-methylformamide (NMF), given in a three times/week oral schedule, closed early because of frequent and occasionally severe toxicity. Eighteen of 41 (44%) cycles of treatment were not completed because of problems with NMF-induced hepatic and gastrointestinal toxicity. Several other reactions occurred, including skin rashes, abdominal pain, and gastritis, which were drug induced. One death occurred on study and was thought to be due in part to NMF toxicity. Further work exploring alternative schedules is needed before phase II studies of oral NMF can be done. [Cancer Treat Rep 70:881–883, 1986]

N-Methylformamide (NMF) is a polar solvent which is currently undergoing phase II evaluation as an anticancer agent in North America and Europe.

In preclinical screening, this compound acted as a differentiating agent in some murine and human tumor cell lines and also exhibited good antitumor activity against three human xenografts (1,2). In toxicology studies performed in mice and beagle dogs, NMF induced hepatotoxicity characterized by reversible elevation of transaminases, alkaline phosphatase, and bilirubin. Microscopic examination of the liver demonstrated varying degrees of hepatocyte necrosis, vacuolization, and subacute hepatitis. Other toxic effects were seen, including reversible neutrophilia and occasional diarrhea, but the liver appeared to be the major target organ for toxicity of the drug in both species.

In phase I trials both the oral and the iv routes of administration have been studied (3–6). Studies of two schedules of oral administration have been reported: daily × 5 (4) and three times/week for 4 of 6 weeks (6).

Toxic effects of the latter schedule included primarily nausea, vomiting, and reversible hepatic enzyme elevations. Less commonly seen were fatigue and hypophosphatemia. The recommended phase II dose for this schedule was 800 mg/m^2/day. Because of interest in the novel class of this drug and acceptable toxicity of NMF when given in an oral schedule, the National Cancer Institute of Canada (NCIC) Clinical Trials Group embarked on three phase II studies of oral NMF in late 1984. The tumor types studied were malignant melanoma, colorectal carcinoma, and malignant glioma. Eligibility criteria for all three trials included: measurable disease, Eastern Cooperative Oncology Group performance status ≤ 3, normal renal function, normal serum bilirubin and transaminase levels (as a measure of hepatic function), and adequate bone marrow function. Patients with melanoma and colorectal carcinoma had to be previously untreated. Glioma patients may have had adjuvant chemotherapy, but NMF was to be the first treatment of relapsed tumors.

MATERIALS AND METHODS

The NMF was given in a starting dose of 800 mg/m^2/day orally three times/week (Monday, Wednesday, and Friday) for 4 weeks. This cycle was repeated every 6 weeks. The daily dose of NMF was diluted in 4 ounces of orange juice and swallowed by the patient. Strict precautions were used to prevent the drug from coming

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TABLE 1.—NMF courses

Dose (mg/m^2)	No. of courses			
			Incomplete	
	Initiated	Completed	Toxicity	Other
600	11	5	5	1
800	30	10	13	7
Total	41	15	18	8

TABLE 3.—NMF (oral) gastrointestinal toxicity*

Dose (mg/m^2)	Total No. of courses	No. of courses with toxicity			
		Grade 1	Grade 2	Grade 3	Total
600	10	1	1	1	3
800	29	1	9	3	13
Total	39	2	10	4	16

*Nausea and vomiting.

into contact with rubber or plastic because of its solvent properties.

While the patients were on study, liver enzymes initially were monitored twice weekly. The NMF was stopped if either the aspartate aminotransferase (AST) or alkaline phosphatase rose to levels more than twice baseline or if the bilirubin rose to 1.5 times the baseline level. After these parameters normalized, the drug was restarted with a 20% reduction of dose. Nausea and vomiting, when moderate or severe, was treated with metoclopramide and/or other antiemetics. If this did not provide relief, then the dose was reduced by 20%.

RESULTS

The trials were opened to accrual in December 1984, and by early March 1985, 22 patients had been registered on the trials.

At about that time, several patients were reported as having failed to complete their first course of therapy because of problems with severe toxicity. In a few, toxicity was such that they were removed from the trial.

Because of this unexpected level of toxicity, trials were closed to general accrual and restricted to three centers. The starting dose was lowered to 600 mg/m^2/day and it was planned to enter five or six patients at this level to assess toxicity and feasibility of continuing the studies. After five patients were entered, a review of toxicity indicated that only one patient had been able to complete a 4-week course of treatment at that dose. The studies were then closed to further accrual, as it was felt that further investigation of the drug in the cooperative group setting was not appropriate.

A total of 27 patients entered the study: 22 at a starting dose of 800 mg/m^2/day and five at a starting dose of 600 mg/m^2/day. In two patients the AST was slightly elevated at the time of entry, but they are included in this summary. The total number of courses initiated and completed at each of these two dose levels is shown in table 1. Nine patients went off study for reasons of toxicity.

As in the phase I trial, the toxic effects seen were primarily nausea, vomiting, and elevation of hepatic enzymes (tables 2 and 3). The pattern of hepatic enzyme changes generally was that of an increase in AST with or without a rise in alkaline phosphatase. In two instances the bilirubin rose as well. The AST rise varied from 1.5 to 18 times the patient's baseline level, with the median maximum value being 133 IU/L. There was no obvious correlation between the occurrence of hepatic toxicity and symptoms of nausea or vomiting, concurrent medication, or the presence of liver metastases (all three glioma patients had grade 2-3 toxicity). The biochemical abnormalities tended to appear in the second or third week of treatment and in all but three patients returned to normal shortly after stopping the NMF.

In two patients the AST remained elevated several weeks after the NMF was stopped. In a third, who received six doses of drug at 800 mg/m^2/day, the AST, alkaline phosphatase, and bilirubin continued to rise despite stopping treatment, and he died on Day 28 of his first cycle. At the time of his death his bilirubin was 325

TABLE 2.—NMF (oral) biochemical hepatic toxicity and toxicity grading

Dose (mg/m^2)	Total No. of courses	No. of courses with toxicity grade—					Total No. of courses with toxicity
		0	1	2	3	4	
600	11	6	4		1		5
800	29	18	6	2	2	1	11

	Toxicity grading*				
	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Bilirubin	< 1.25 × N	1.25-2.5 × N	2.6-5.0 × N	5.1-10.0 × N	> 10.0 × N
SGOT	< 1.25 × N	1.25-2.5 × N	2.6-5.0 × N	5.1-10.0 × N	> 10.0 × N
Alkaline phosphatase	< 1.25 × N	1.25-2.5 × N	2.6-5.0 × N	5.1-10.0 × N	> 10.0 × N

*N = normal.

μmols/L (normal, 2–18), AST was 116 IU/L (normal, 10–35), and alkaline phosphatase was 774 IU/L (normal, 30–110). A liver biopsy showed moderate periportal fibrosis and inflammation, intracanalicular biliary stasis, and occasional hepatocytes with clear nuclei. At autopsy the liver was extensively replaced by tumor, but there was no extrahepatic biliary obstruction. His death was felt to be due to a combination of drug toxicity and malignancy.

Other toxic effects noted on study and attributed to NMF included a macular, erythematous rash (four patients), weakness (three), abdominal pain or heartburn (four), and somnolence (two). One patient with abdominal pain underwent endoscopy and was found to have a moderately severe antral gastritis which, in the absence of any other cause, was felt to be due to NMF.

It should be noted that toxicity was not universal. Ten courses of treatment at the 800-mg/m^2 level and three at the 600-mg/m^2 level were administered without any symptomatic or biochemical changes. An examination of several factors including tumor type, sites of disease, presence of hepatic metastases, and concomitant medications did not provide an explanation for the individual variability in the degree of toxicity experienced.

Twenty-one patients were evaluable for response (melanoma, nine; colorectal, nine; and glioma, three) and no responses were seen. The majority had disease progression on therapy, and nine remained stable for periods ranging from 6 to 30 weeks.

DISCUSSION

These three phase II studies using oral NMF in a three times/week-schedule closed early because of a higher than anticipated incidence of toxic effects, both at the 800- and 600-mg/m^2/day level. Eighteen of 41 courses (44%) could not be completed because of toxicity and nine of the 27 patients entered on the studies were taken off trial because of drug-related toxic effects. No single factor, including concomitant medication or hepatic metastases, seemed to predict which individuals would experience more toxicity than others.

In conclusion, the doses and schedule of oral NMF we used produced a level of toxicity which made continued phase II evaluation unacceptable. The degree of patient-patient variability in toxicity which we observed may indicate that future oral administration schedules will need to be based on gradual dose escalation to individual tolerance. More pharmacokinetic and bioavailability information is needed to define the optimal dose and schedule of oral NMF to take into phase II trials.

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Aminothiadiazole: An Antineoplastic Thiadiazole[1]

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Several thiadiazoles are currently being clinically evaluated as cancer chemotherapeutic agents in the United States and Europe. These agents are thought to be cytotoxic through their inhibition of de novo purine biosynthesis secondary to their inhibition of inosine monophosphate dehydrogenase (IMPD), a regulatory enzyme of the de novo purine pathway. Aminothiadiazole (ATDA) was initially evaluated in the late 1950s, re-entered phase I trials in 1975, and has recently progressed to phase II trials. With the introduction of tiazofurin in the United States and its selenium-containing analogue selenazole in Europe, a re-evaluation of the oldest thiadiazole, ATDA, is warranted.

Preclinical Antitumor Activity

The antitumor activity of ATDA and its derivative, ethylamino-1,3,4-thiadiazole (EATDA), was first reported by Oleson and his associates in 1955 (1). These authors demonstrated pronounced activity against melanoma S-91, glioblastoma 8110, and 6C3HED lymphosarcoma. Burchenal and Dagg (2) reported EATDA to be active against leukemia 8174. Troy et al (3) reported marked inhibition of a glioblastoma, a melanoma, and a lymphosarcoma, and varying inhibition against mammary adenocarcinoma, sarcoma 180, and P1534 leukemia. These authors demonstrated reversal of tumor inhibition and toxicity by nicotinamide and nicotinic acid (3). Ciotti et al (4) demonstrated ATDA's chemotherapeutic effect in L1210 leukemia in vivo, and confirmed the inhibitory activity of nicotinamide.

Further testing of ATDA by the National Cancer Institute (NCI) (5) revealed some schedule dependency against L1210 leukemia with the greatest increased life span (ILS) attained with the schedule of every 3 hours on Days 1, 5, and 9. In the NCI screen, ATDA was also shown to be active against P388 leukemia in mice, the Walker 256 implanted intramuscularly in rats, and the Lewis lung carcinoma in mice (table 1). ATDA was inactive in B16 melanoma, adenocarcinoma 755, ependymoblastoma, and the subcutaneously implanted mouse sarcoma 180 (5). These results are similar to those obtained with tiazofurin in the NCI screen, except that the latter was considerably more active against the Lewis lung carcinoma (6).

Mechanism of Action

Following the discovery of antibiotics, cancer chemotherapeutic research was directed towards a search for quantitative biochemical differences between tumor and host. It was in this atmosphere that ATDA was reported to exert antitumor activity (1), and the initial report of tumor inhibition was closely followed by reports that nicotinamide and nicotinic acid reversed both the antitumor activity and toxicity of ATDA (3,7). The subsequent evaluation of EATDA, an analog of ATDA, by Krakoff and Magill (8) revealed a marked elevation of serum uric acid in man coincident with administration of the drug.

Early mechanism studies centered on the observations of nicotinamide reversal of antitumor activity and increased production of uric acid. In clinical studies, Krakoff et al (9-11) demonstrated that EATDA's uricogenic effect could be blocked by nicotinamide as well as by the purine synthesis inhibitors 6-diazo-5-oxo-L-norleucine (DON), *O*-diazo-acetyl-L-serine (azaserine), 6-mercaptopurine, and allopurinol. Using radiolabeled purine precursors of uric acid, these authors established that EATDA caused an increase in de novo uric acid synthesis (9). Experiments conducted by Krakoff (12) in the chick embryo suggested that ATDA initiates the uricogenesis by blocking nucleotide incorporation into nucleic acid. Stimulation of de novo purine synthesis results, and the block at the level of IMPD leads to overaccumulation of uric acid. The results of Duggan et al (13) are consistent with this hypothesis. Mediation through the de novo rather than the salvage pathway was confirmed by the observation that uric acid production was increased following ATDA administration in patients with hypoxanthine guanine phosphoribosyl transferase deficiency (14).

At the molecular level, an exchange reaction has been reported between thiadiazole compounds and the nicotinamide moiety of nicotinamide adenine dinucleotide (NAD) (4). To clarify the mechanism of action of the

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TABLE 1.—Preclinical antitumor activity of ATDA

Tumor	Optimal dose of ATDA (mg/kg/day)	Route	Optimal schedule	ILS (%)*	S/T*
L1210	128	Ip	Every 3 hrs on Days 1, 5, and 9	94	0/10
P388	15-50	Ip	Days 1-10	90	
Lewis lung	200	Ip	Every 4 days (Days 1, 5, 9, 13, and 17)	74	
Walker 256 (im implanted)	30	Ip	Every day on Days 3-6	†	

*ILS = increased life span; S/T = No. of survivors/total No. of animals.
†Tumor weight inhibition = 92%.

thiadiazoles, the effects of the analog 2,2′-(methylenediimino)bis-1,3,4-thiadiazole were studied in vitro using monolayer cultures of growing cells of the mouse cell line BALB/3T3 (15). At concentrations < 50 mM, the synthesis of DNA and RNA was inhibited equally and concomitantly by the compound. Inhibition was reversed by removal of the compound, or addition of guanosine or deoxyguanosine, and competitively prevented by equimolar nicotinamide. Results of these experiments suggested that activity was due to inhibition of GMP biosynthesis at the level of conversion of inosine monophosphate (IMP) to xanthosine 5′-phosphate. Nelson et al (16) provided further support for the mechanism by studying the effects of ATDA on purine and pyrimidine nucleotide pools of L1210 ascites cells in vivo. Their conclusion that ATDA or a metabolite inhibits the conversion of IMP to guanosine monophosphate was reinforced by the observation that mycophenolic acid, a known inhibitor of the conversion, produced effects on ribonucleotide pools similar to those produced by ATDA. Furthermore, the effects of ATDA on nucleotide pools were prevented by simultaneous administration of nicotinamide. Thus, since nicotinamide is known to prevent or reverse the antitumor activity of ATDA, it is concluded that its mechanism of antitumor activity is through inhibition of guanosine monophosphate synthesis.

To investigate the role of metabolites in ATDA's mechanism of action, Nelson et al (17) synthesized two derivatives of ATDA: (*a*) an analog of NAD (ATDA adenine dinucleotide); and (*b*) the mononucleotide of ATDA. Both were shown to be potent inhibitors of L1210 IMPD; the NAD analog was a pseudoirreversible inhibitor of IMPD and the ATDA mononucleotide was a reversibly competitive inhibitor. Following administration of labeled ATDA to mice bearing L1210 cells, formation of an ATDA mononucleotide was demonstrated; the NAD analog was not detected in vivo. Nicotinamide prevented formation of the mononucleotide in vivo (17).

Hence, there is evidence that the active metabolite of ATDA may be the mononucleotide. This contrasts with the demonstrated mechanism of action of tiazofurin, another IMPD inhibitor currently in clinical development. Jayaram et al (18) have shown that tiazofurin is also metabolized to a mononucleotide which inhibits IMPD. However, tiazofurin is further anabolized to an NAD analog [thiazole adenine dinucleotide (TAD)] which is a more potent inhibitor of IMPD than the mononucleotide ($K_i = 0.5$ vs 400 mM) (19). TAD levels correlate well with the cytotoxicity of tiazofurin in vivo (20), and resistance to tiazofurin is associated with absence of TAD formation (21). These differences may account for the dissimilar patterns of toxicity in phase I studies of the thiazole analogs.

In summary, results indicate that the cytotoxicity and presumably the antitumor effects of ATDA are the result of inhibition of IMPD. Whether this blockade is also responsible for the observed uricogenic activity of the thiadiazoles is not yet known. The uricogenic effect may be a response by the cell to the blockade of IMPD—an increased synthesis of purines de novo with consequent accumulation of IMP, which is then eliminated as uric acid. The observation that nicotinamide reversed both the antileukemic and the uricogenic activity of 2-ethylaminothiadiazoles (4,10) is consistent with this hypothesis. Mechanistic differences between the IMPD inhibitors currently in clinical trials support their separate clinical evaluation.

Pharmacology

Information concerning the pharmacokinetic behavior of ATDA is limited. El Dareer et al (22) studied the distribution and metabolism of ^{14}C-ATDA in mice, dogs, and monkeys. In mice injected ip with 300 mg/m^2 of ATDA, serum levels decrease with a half-life of 2.9 hours. Although El Dareer et al did not completely characterize the pharmacokinetics in dogs, Lu and Loo (23) demonstrated a terminal plasma half-life of 13 hours in four dogs following iv administration of ATDA at 200 mg/m^2. Similar results were obtained with a dose of 500 mg/m^2, suggesting that the pharmacokinetics of ATDA are not dose-dependent over this dose range. Those investigators also noted that the pharmacokinetics of ATDA were not altered appreciably with the simultaneous iv administration of allopurinol at 30 and 60 mg/kg.

In mice, 73% of the total ip dose was excreted in the urine as unchanged ATDA in the first 24 hours (22). The mean total clearance of ATDA in the dog was 0.7 ml/kg/minute (23). The dog excreted unchanged drug through the first 12 hours; urinary metabolites were identified at 24 and 48 hours (22). At autopsy 5 hours after dosing in the dog, > 75% of the administered radioactivity was retained in the body; highest amounts were located in liver, kidney, lung, and stomach.

Ackerly et al (24) developed a thin-layer chromatography/high-pressure liquid chromatography assay to simultaneously quantitate ATDA, uric acid, allopurinol, and nicotinamide in human serum. A terminal half-life of 2.2 hours was observed for ATDA administered at 50 mg/m^2 to six patients. Serum levels and elimination half-life were comparable in patients treated concomitantly with allopurinol and those treated only with ATDA.

Clinical Trials

The early clinical studies of the 2-substituted thiadiazoles emphasized metabolic effects and mechanism of action and were conducted by Krakoff and co-workers (8,9,11). In their earliest study (8), EATDA was administered orally at doses of 2-6 mg/kg/day. Toxic effects were limited to severe glossitis, which occurred in 4-6 days at a dose of 4 mg/kg. It was in this study that the uricogenic effect was first noted. When ATDA was administered orally to four patients at a dose of 50 mg, results were reported to be identical to those observed with EATDA (9). The uricogenic and toxic effects of both ATDA and EATDA were blocked or reversed by the administration of equal or greater doses of nicotinamide, whereas the purine synthesis inhibitors DON and azaserine blocked only the uricogenic effects (8). Later studies showed that allopurinol also selectively blocked the uricogenic effect (11).

Phase I

In 1975, ATDA was re-evaluated at the University of Vermont in a phase I trial. Sixteen patients were treated with ATDA; five of the 16 received ATDA alone and the remaining 11 received ATDA in combination with allopurinol. As predicted by the early metabolic studies by Krakoff and Magill (8), the five patients who were treated with only ATDA developed hyperuricemia. Concomitant treatment with allopurinol prevented the development of hyperuricemia. The dose-limiting toxicity of stomatitis was common to both groups.

A review of the ATDA preclinical data revealed less toxicity and enhanced therapeutic efficacy when ATDA was administered every 4 days to mice bearing L1210 leukemia. Interest in ATDA was renewed and another phase I trial was initiated in 1980 (25). To block ATDA's uricogenic effect, allopurinol was administered daily from 1 day before through 2 days after ATDA. In this trial, mucosal toxicity, mainly oral, was consistently limiting but was related to frequency of administration, not to size of dose. It occurred regularly at all doses from 12 to 200 mg/m^2 given daily or every 3 days, but was infrequent with weekly administration of doses of 125 mg/m^2 (26). The only other reported toxicity was moderate nausea and vomiting, which occurred at the higher doses. Partial responses were seen in two patients with non-small cell lung cancer. The recommended phase II dose was 125 mg/m^2 weekly. Dose escalation is appropriate for most patients if mucositis is mild.

Phase II

The evaluation of ATDA has been completed in only a few tumor types. In non-small cell lung cancer, studies at Vermont Regional Cancer Center (25) and the Illinois Cancer Council (27) yielded only two partial responses among 62 patients treated (3%). In colon cancer, the latter group also performed a phase II study. Although only one of the 71 patients had a documented partial response, almost half the patients had stabilization of their disease for extended periods (28). A study to confirm these results has been completed by the Eastern Cooperative Oncology Group. These data suggest that combinations involving ATDA should be explored.

Current and Future Phase II Studies

Although there are no data for ATDA, tiazofurin has been shown to be synergistic with 5-FU in some models,[5] and the drug inhibits uridine transport in human red blood cells (29). Since red blood cells cannot form the active metabolite TAD, and since IMPD inhibition increases intracellular phosphoribosylpyrophosphate (PRPP) levels (30), two potential mechanisms for the modulation of 5-FU activation are suggested: (*a*) diminished transport of the competing substrate, uridine; and (*b*) increased availability of PRPP. These mechanisms suggest that the optimal expression of synergy should be critically dependent on dose and schedule (31). Before conducting a clinical study of ATDA and 5-FU, further work in vitro and in vivo should be performed to clarify these issues. Similarly, the synergy of tiazofurin and cisplatin noted in vivo requires further preclinical exploration (32).

The evaluation of ATDA as a single agent is continuing. Additional studies are under way in colon and non-small cell lung cancers, while the drug is also being evaluated in breast cancer, advanced pelvic malignancies, and non-Hodgkin's lymphoma. A broader phase II evaluation in a larger number of tumors is clearly desirable, especially given the responses noted in highly resistant tumors. Evidence of activity in a given disease should lead to the development of combinations in which ATDA may be synergistic with other agents. Such combinations may be designed empirically, or with a biochemical/biological rationale following in vitro and in vivo studies of drug interaction. Integration of the parallel development of tiazofurin and ATDA should facilitate exploration of the role of IMPD inhibition in cancer therapy.

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[5] Data on file, Developmental Therapeutics Program, Division of Cancer Treatment, NCI, 1984.

Toxic Effects

The phase II studies to date have confirmed and expanded the toxicity data from the phase I trials. Grade 2–3 mucositis occurs in 4%–20% of patients, and nausea and vomiting occurs in 8%–10%. In addition, anemia (often requiring transfusion) has been noted in 9% of patients, and eight patients have been described as having a syndrome of pericarditis similar to that described in the phase I studies of tiazofurin (33). Symptoms of pericarditis resolve rapidly with nonsteroidal anti-inflammatory agents, and no long-term sequelae have been reported. It is of interest that the incidence and severity of toxic effects differ for these two inhibitors of IMPD. While a syndrome of asthenia, fatigue, and pericarditis was dose-limiting in one of the phase I studies of tiazofurin (33), it was not prominent in other studies (34,35), which had myelosuppression (34) or neurotoxicity (36) as the predominant side effects. Mucositis was not prominent in any of the studies.

CONCLUSIONS

ATDA was the first thiadiazole to be evaluated in the clinic as an antineoplastic agent. Its administration leads to inhibition of IMPD, presumably after anabolism to the mononucleotide, and to increased production of uric acid. Evaluation of several schedules in phase I studies revealed that weekly administration of 125 mg/m^2 with concomitant allopurinol was safe and well-tolerated. Differences between the toxic effects of ATDA and those of tiazofurin, which is just entering phase II trials, suggest that metabolic effects other than inhibition of IMPD may be important. Early results from phase II studies of ATDA show that it is inactive in non-small cell lung cancer. Trials in other tumor types are in progress. The parallel development of these thiadiazoles in the clinic, together with continuing basic research on their cellular mechanisms, will contribute to an understanding of appropriate biochemical targets for successful antimetabolite therapy.

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BRIEF REPORTS

Etoposide and High-Dose Cisplatin in Good-Risk Patients With Advanced Squamous Cell Carcinoma and Adenocarcinoma of the Lung[1]

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Fifty patients with advanced squamous cell carcinoma and adenocarcinoma of the lung having good prognostic features (performance status ≤ 2; weight loss ≤ 10%; age ≤ 70 years; absence of brain metastases; and no prior treatment) were treated on an outpatient basis with etoposide (120 mg/m^2 on Days 1, 3, and 5) and cisplatin (60 mg/m^2 on Days 1 and 2) every 21–28 days. One complete response and 14 partial responses were observed, with a median duration of response of 170 days and an overall median survival of 230 days. Toxicity was generally mild. Despite the high-dose cisplatin employed and the choice of patients with favorable prognostic factors in this study, results of this therapy remain disappointing. [Cancer Treat Rep 70:891–892, 1986]

The exact role of chemotherapy in non-small cell lung cancer (NSCLC) is still unclear. Recently, the combination of cisplatin with either etoposide or vindesine seems to have induced encouraging improvement, at least in terms of remission rate (1). Nevertheless, the results reported by various investigators are often inconsistent, probably due to the different type of patient selection. In fact, several prognostic factors (ie, performance status, weight loss, and prior treatment) have been recognized to be a determinant for response and survival regardless of treatment (2). Based on data supporting superiority, at least in terms of response duration, of high-dose with respect to low-dose cisplatin in the treatment of NSCLC (3), we have undertaken a phase II study employing a combination of etoposide and high-dose cisplatin administered as an outpatient regimen in patients with adenocarcinoma and squamous cell carcinoma of the lung. The study was carried out in patients selected according to the known prognostic features.

PATIENTS AND METHODS

From September 1982 to August 1984, 50 patients with histologically and/or cytologically confirmed squamous cell carcinoma or adenocarcinoma of the lung were entered in the study. All patients had stage III disease as established by American Joint Committee for Cancer Staging and End Result Reporting criteria (4). Criteria of eligibility were: age ≤ 70 years; no prior chemotherapy or radiotherapy; performance status ≤ 2 [Eastern Cooperative Oncology Group (ECOG) scale]; weight loss ≤ 10% in the last 3 months; and no brain metastases. Patients were required to have measurable or evaluable disease according to World Health Organization (WHO) criteria (5), serum creatinine ≤ 1 mg/100 ml, creatinine clearance > 70 ml/minute, leukocyte count ≥ 4000/mm^3, platelet count ≥ 100,000/mm^3, and hemoglobin ≥ 10 g/100 ml. Patient characteristics are shown in table 1. Pretreatment evaluation consisted of complete history and physical examination, laboratory evaluation including 12-channel biochemical profile, and chest tomography or computerized chest tomography. Radionuclide bone and brain scans and hepatic ultrasonography were performed only if history, physical examination, or laboratory findings indicated possible metastatic involvement. Patients were treated on an outpatient basis with etoposide (120 mg/m^2 on Days 1, 3, and 5 iv over 30 minutes), cisplatin (60 mg/m^2 on Days 1 and 2 with 2-hour hydration) (6), and furosemide-enforced diuresis every 3–4 weeks according to hematological recovery. Chemotherapy was continued up to a minimum of six cycles or until disease progression or unacceptable toxicity. Patients were evaluated for response according to WHO criteria (5) if they had com-

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TABLE 1.—Patient characteristics

Characteristic	No. of patients (%)
Total	50
Median age in yrs (range): 61 (35–70)	
Sex	
Male	43 (86)
Female	7 (14)
Histology	
Adenocarcinoma	17 (34)
Squamous cell carcinoma	33 (66)
Stage	
M_0	30 (60)
M_1	20 (40)
Performance status (ECOG)	
0	26 (52)
1	17 (34)
2	7 (14)
Weight loss	
0	40 (80)
≤ 10%	10 (20)

pleted at least two cycles of therapy. According to Eagan et al (7), the resolution of atelectasis was accepted as a valid criterion for tumor response. All records were reviewed by three investigators. Both survival and time to disease progression were recorded from the beginning of treatment.

RESULTS

Fifty patients were entered in the study. Of these patients, four received an inadequate trial: one had early death unrelated to treátment after one cycle of therapy; one had an abscess in the tumor mass requiring withdrawal of treatment; one had a major protocol violation; and one had spontaneous discontinuation of treatment due to gastrointestinal intolerance after one course.

Fifteen of 50 patients (30.0% ± 12.7%; 95% confidence limits) achieved complete (CR) or partial (PR) response (one CR and 14 PRs). The CR was achieved in a patient with measurable bilateral lung nodules of squamous histology and lasted 9 months. PR was achieved in ten patients with measurable disease and in four with evaluable disease (two of four had complete resolution of major atelectasis in which nonconcomitant antibiotics or bronchodilators were used). Response occurred in lung, lymph nodes, and liver in 14, two, and one patient, respectively. Toxicity was evaluated according to WHO criteria. Nausea and vomiting and alopecia (grade III) were almost universal (80% and 70% of patients, respectively). Bone marrow toxicity was generally not severe. Seven patients had grade II leukopenia, two had grade III, and three had grade IV; three patients had grade IV thrombocytopenia. Five patients had mucositis (10%). A transient rise in serum creatinine (grade I in four patients and grade II in one) was observed. Ototoxicity, defined as tinnitus and/or hearing loss, was observed in six of 50 patients (12%). Peripheral neuropathy with decrease in deep tendon reflex and appearance of paresthesias was observed in six of 50 patients (12%).

CONCLUSION

In this trial, an effort has been made to select patients with homogeneous features to avoid, as far as possible, bias selection, to obtain more comparable data, and to choose a good-risk population drawing the best benefit from combination chemotherapy. Nevertheless, our results, despite the high-dose cisplatin regimen used and the good-risk population selected for the trial, are not different in terms of response rate and survival from those reported by other investigators (8–10). In our regimen, high-dose cisplatin has been employed on an outpatient basis, giving the total dose of 120 mg/m^2 divided in 2 consecutive days. In this way, we obtained comparable efficacy but better patient compliance and less toxicity. In particular, an unreversible increase in serum creatinine has never been observed. These disappointing results, even in good-risk patients, together with the subjective intolerance achieved with the combination of high-dose cisplatin and etoposide, support the need for new drug investigations in patients with NSCLC.

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Cisplatin Plus Etoposide as Second-Line Treatment in Advanced Ovarian Carcinoma[1]

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We have treated 25 consecutive patients with advanced ovarian carcinoma with cisplatin (100 mg/m^2 iv on Day 1) and etoposide (100 mg/m^2 orally on Days 1-3) every 3 weeks. All patients had failed previous treatment with doxorubicin and cyclophosphamide and had bulky abdominal disease. Complete response was achieved in one of 25 patients (4%), partial response was achieved in nine of 25 patients (36%), and stable disease was obtained in six additional patients (24%). Median time to disease progression was 6.8 months. Toxicity was acceptable. Cisplatin and etoposide is an effective second-line treatment of ovarian carcinoma. [Cancer Treat Rep 70:893-895, 1986]

Cisplatin (CDDP) has proven to be the single most effective cytotoxic agent for the treatment of advanced ovarian carcinoma in patients not previously treated as well as in patients with previous exposure to chemotherapy (1-4). In particular, when used as a single agent in second-line treatment, this drug is able to achieve a 30%-50% response rate (1,3,4). A recent review of etoposide (VP16) in advanced refractory ovarian carcinoma (5) reported poor results (12% response rate among 101 patients), but experimental studies and clinical trials (6,7) have demonstrated a therapeutic synergism between CDDP and VP16 in various tumors. Based on the results of these studies, we undertook a phase II study on the effectiveness and toxicity of the CDDP plus VP16 combination in patients with stage III-IV ovarian carcinoma.

PATIENTS AND METHODS

Twenty-five consecutive patients with bulky advanced ovarian carcinoma were entered in the study between October 1981 and March 1985. All patients are currently evaluable, with a median follow-up of 8 months (range, 3-19). Moreover, all patients had failed previous therapy with doxorubicin plus cyclophosphamide (AC) administered for at least three cycles [doxorubicin (50-70 mg/m^2) and cyclophosphamide (700-1000 mg/m^2) for each cycle]; one of them had also been pretreated with CDDP. Patient characteristics are summarized in table 1. Treatment consisted of CDDP at a dose of 100 mg/m^2 iv on Day 1 (administered with usual hydration modalities) and VP16 at a dose of 100 mg/m^2 orally on Days 1-3, recycled every 3 weeks. All patients received at least two courses of therapy and were evaluated for response and toxicity according to World Health Organization criteria (8). In case of myelosuppression the drug dose was reduced as follows. If the peripheral leukocyte count was 3999-3000 and/or the platelet count was 120,000-100,000/mm^3, the CDDP and VP16 doses were 100% and 50%, respectively; for lower values the treatment was delayed 1 week.

RESULTS

Complete remission (CR) was achieved in only one patient (4%) and partial remission (PR) was achieved in nine of 25 patients (36%). Moreover, six additional patients, one of whom was also pretreated with CDDP, had stable disease (24%). After six cycles of therapy, three women (one with CR and two with PR) were subjected to a surgical look which confirmed that the clinical CR was indeed a pathological CR, and allowed a partial debulking in the two other patients. The characteristics of responsive patients are shown in table 2. We observed that seven of 13 (54%) patients who had previously responded to AC treatment also responded to CDDP plus VP16. On the other hand, only three of 12 (25%) patients who had not previously responded to AC showed response to CDDP plus VP16. This observation indicates that in some patients AC-resistant tumors are not cross-resistant to CDDP plus VP16. The median time to disease progression was 6.8 months. The median survival was 8 months for all patients and 6 months for nonresponders; it has not yet been reached for responders. The actuarial analysis of survival with the log-rank test method of Cox modified by Mantel (9) showed a significant difference between responders and nonresponders ($P < 0.025$; chi-square, 6.3). The tumor

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TABLE 1.—Characteristics of 25 evaluable patients

Characteristic	No. of patients
Median age in yrs (range): 53 (31-67)	
Stage	
III	18
IV	7
Histology	
Serous	17
Mucinous	3
Undifferentiated	4
Endometrioid	1
Grade	
I	1
II	11
III	9
Not determined	4
Performance status (Karnofsky)	
> 70	16
< 70	9

grade did not significantly influence response and survival although there was a trend in favor of grade I-II patients.

Grade III leukopenia (wbc count, 1000-1900/mm^3) was observed in only four of 123 cycles administered, and grade II thrombocytopenia (platelet count, 50,000-74,000/mm^3) was observed in 1% of cycles. Grade II anemia (hgb, 8-9.4 g/100 ml) occurred in five patients and grade III anemia (hgb, 6.5-7.9 g/100 ml) occurred in three. Transient elevation of BUN occurred in two patients. Nausea, vomiting, and alopecia were universal. Two women developed excitement and tremors during CDDP administration.

DISCUSSION

Although ovarian cancer is a very chemosensitive tumor, second-line treatments obtain only limited results. In fact, Briscoe et al (10), combining doxorubicin with CDDP, reported only a 30% response rate in 24 pretreated patients and Neijt et al (11) confirmed the dismal results achievable in patients with previous exposure to alkylating agents. Nevertheless, CDDP is the most effective agent in pretreated patients. Piver et al (12) demonstrated that this drug was also active in women with previous exposure to CDDP provided the dose was increased. A remarkable synergism between CDDP and VP16 was demonstrated in experimental models and in clinical studies (6,7), and the capacity of VP16 to enhance the CDDP activity could also be used in heavily pretreated ovarian cancer. In fact, Barlow and Lele (13) treated ten patients who had previously received various drugs including CDDP with CDDP plus VP16 and observed four PRs and three patients with stable disease in eight evaluable patients.

Our results confirm that CDDP plus VP16 is an effective combination in pretreated advanced ovarian cancer but could not indicate a synergism between the two drugs. In fact, only randomized trials can demonstrate a possible benefit from the addition of VP16 to CDDP in such patients.

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TABLE 2.—Characteristics of responding patients*

Patient No.	Age (yrs)	Karnofsky performance status	Histology	Stage	Grade	Response to AC	Response to 2nd line	Time to disease progression (mos)	Survival (mos)
1	60	70	S	III	III	Pro	PR	4	6
2	31	90	M	III	II	Pro	PR	6	8
3	54	50	S	IV	ND	PR	PR	9	10
4	52	100	S	III	ND	PR	PR	5	8
5	53	70	S	III	I	CR	PR†	8	15+
6	50	100	S	III	II	PR	PR†	12	19+
7	49	70	S	III	II	Pro	CR†	15+	15+
8	67	100	S	III	II	CR	PR	5	14+
9	52	70	E	III	II	PR	PR	4+	4+
10	59	60	U	III	II	PR	PR	6+	6+

*S = serous; M = mucinous; E = endometrioid; U = undifferentiated; ND = not determined; and Pro = disease progression.

†Pathological evaluation.

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BRIEF REPORTS

Treatment of Advanced Gastric Carcinoma With Mitomycin and Doxorubicin[1]

David Dalley,[2,*] Charles Erlichman,[3] and Sheldon Fine[3]

Thirty patients with advanced gastric carcinoma were treated with doxorubicin combined with mitomycin; only four (13%) responded. Myelosuppression was the major toxic effect; there was one toxic death. The overall median survival was 14 weeks (range, 2–42), which is no different from that reported for untreated patients. The combination of doxorubicin and mitomycin alone cannot be recommended for use in patients with advanced gastric carcinoma. [Cancer Treat Rep 70:897-898, 1986]

Studies utilizing the combination of 5-FU, doxorubicin, and either mitomycin (FAM) (1–3), semustine (4), or carmustine (5) have reported response rates of 25%–55%, with a median survival of 6 months. The dose of doxorubicin used in these combinations was relatively low (30–40 mg/m^2 every 28 days); if the dose of doxorubicin is important, as suggested by the use of high-dose doxorubicin alone in gastric cancer (6), then a higher dose given at more frequent intervals might be expected to improve response and survival. To study the effect of a higher dose of doxorubicin, 5-FU was omitted and the combination of doxorubicin plus mitomycin was evaluated.

PATIENTS AND METHODS

Thirty patients with advanced gastric adenocarcinoma with either metastatic or surgically unresectable disease were studied. All patients had at least one area of measurable or evaluable disease; the predominant sites of disease as well as the principal patient characteristics are shown in table 1. Patients were excluded from entry if they had had prior chemotherapy, extensive radiation therapy within the previous 4 weeks, pre-existing serious systemic disease, previous carcinoma (excluding basal cell carcinoma or completely excised squamous cell carcinoma of skin), performance status $< 30\%$ on the Karnofsky scale (7), or life expectancy < 2 months.

All patients received at least one course of the combination consisting of doxorubicin (50 mg/m^2 iv on Day 1 given every 3 weeks) and mitomycin (10 mg/m^2 iv on Day 1 repeated every 6 weeks). When necessary, drug doses were adjusted according to the degree of myelosuppression observed with previous courses and the dose of doxorubicin was modified if serum bilirubin was elevated.

Response was assessed after 6 weeks and was in accordance with World Health Organization criteria (8). The durations of response and survival were calculated from the start of chemotherapy.

RESULTS

Of the 30 patients, only 22 received more than one course of therapy. Of the eight patients who received only one course of therapy, one patient died of septicemia which occurred during a period of profound neutropenia, one died of myocardial infarction, and another died of a perforated colonic diverticulum; the other five patients developed progressive disease.

The overall response rate was only 13%; two patients achieved complete response (CR) and two achieved partial response (PR). All of the responders had a performance status $\geq 60\%$. The durations of response were 17 and 28+ weeks for CR and 18 and 26 weeks for PR. The chance that a true response of 25% is being missed is $< 5\%$ (Gehan) (9).

The median survival for the whole group was only 14 weeks (range, 2–42). The survival times of the patients with CR were 21 and 28+ weeks and those for the patients with PR were 22 and 37 weeks.

Myelosuppression was the most important side effect. Following the first course of chemotherapy, the median neutrophil count nadir was 0.6×10^9/L (range, 0.08–2.7) and the median platelet count nadir was 165×10^9/L (range, 37-370); no abnormal bleeding was observed. Of the 22 patients who received more than one course of therapy, 16 (73%) required dose reductions because of

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TABLE 1.—Patient characteristics

Characteristic	No. of patients
Total	30
Male:female	19:11
Median age in yrs (range): 56 (35–75)	
Performance status (%)*	
≥ 70	15
50–60	11
30–40	4
Predominant measurable sites of disease	
Liver	10
Lymph nodes	7
Pelvis	4
Stomach	4
Lung	2
Bone	1
Skin	1
Surgery performed	
Unresected	21
Partial gastrectomy	7
Total gastrectomy	2

*Karnofsky scale (ref 7).

severe myelosuppression. Other toxic effects included alopecia (100%), severe nausea and vomiting (10%), and mucositis (3%).

DISCUSSION

The results of this trial were disappointing, especially since the overall survival was no different from that previously reported for untreated patients (10). The poor results obtained may, in part, be due to patient selection, since a large proportion (73%) had bulky unresected tumors and several had a relatively low performance status. The omission of 5-FU from the FAM regimen may have been an important factor. In a randomized study, the Eastern Cooperative Oncology Group found that FAM was superior to doxorubicin plus mitomycin (response rate, 39% vs 29%; survival, 29½ vs 19 weeks, respectively) (11). Response was also lower in patients with poor performance status. Killen et al (12), in a retrospective analysis of 18 randomized trials, point out that regimens containing both 5-FU and doxorubicin have the highest response rates. The importance of doxorubicin is also supported by the Gastrointestinal Tumor Study Group trial comparing two combinations with and without doxorubicin; the doxorubicin-containing combination resulted in a significantly improved survival (13). This improved survival, however, was not found by the North Central Cancer Treatment Group study in which 5-FU alone was compared with 5-FU plus doxorubicin or FAM (14). The reason for this conflicting evidence may, in part, be due to inherent biologic heterogeneity as discussed by Killen et al (12).

In conclusion, the combination of doxorubicin and mitomycin without 5-FU cannot be recommended for use in patients with advanced gastric carcinoma.

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BRIEF REPORTS

High-Dose Mitoxantrone in Metastatic Breast Cancer: A Phase I-II Trial[1,2]

Jane M. Leiby,[3,*] Donald V. Unverfurth,[3] and James A. Neidhart[3,4]

Eighteen women with metastatic breast cancer entered a phase I-II study of high-dose mitoxantrone (MXT). They were heavily pretreated (mean, 2.3 prior regimens) with drugs including both doxorubicin (14 patients) and MXT at usual doses (three). Doses studied were 16, 20, 25, and 30 mg/m^2, repeated every 3-4 weeks. Marked leukopenia was seen at all dose levels (four episodes of infection were successfully treated). Fatigue and malaise were the most common nonhematologic toxic effects. At 25 mg/m^2, six of 13 courses resulted in severe fatigue and malaise. MXT is tolerated at doses up to 25 mg/m^2 every 3-4 weeks. Cardiac function must be closely monitored. No responses were seen in these heavily pretreated patients. [Cancer Treat Rep 70:899-901, 1986]

Despite its reputation as a responsive tumor, metastatic breast cancer is uniformly fatal (1). Further progress in this malignancy demands new treatment modalities or new applications of the old.

A dependence of response on dose for most antineoplastic agents including mitoxantrone (MXT) has been supported by preclinical evidence (2,3). Response rates to chemotherapy may depend on dose in several solid tumors, including breast cancer (4-6). MXT is an active drug against breast cancer, with a dose-limiting toxic effect of myelosuppression (7,8). The goal of this phase I-II trial was to find the maximum tolerated dose of MXT in terms of nonhematologic toxicity.

MATERIALS AND METHODS

Women with recurrent breast cancer who failed at least one previous regimen of cytotoxic chemotherapy were eligible if they had a Zubrod performance status (PS) of 2 or better or had a PS of 3 because of bone pain due to metastatic disease. All patients gave informed consent. Patients who had received MXT at usual doses (12-14 mg/m^2) were eligible for this dose-finding study. Cardiac function was normal prior to therapy as assessed by gated radionuclide scan. Patients were followed during therapy with systolic time intervals (STI). Other pretreatment studies included tumor measurement, blood cell count, liver and renal function tests, chest x-ray, and radionuclide bone and liver scans.

MXT was given as a short iv infusion every 3-4 weeks as permitted by recovery. The starting dose of 16 mg/m^2 was escalated by 5 mg/m^2 when three patients at the previous level had been treated and recovered. Blood cell counts were done weekly until grade 3 myelosuppression, then every 3 days until recovery. Renal and liver function tests were repeated prior to each course.

RESULTS

Eighteen patients were treated; the median age was 57 years (range, 20-70). Fifteen patients had a PS of 2 or better. The mean number of prior cytotoxic regimens was 2.3 per patient. Fourteen patients had had doxorubicin (DOX), with a median total dose of 322 mg/m^2 (range, 95-704); three had had MXT, with total doses of 24, 36, and 56 mg/m^2. Nine patients each had received radiation therapy and/or hormonal manipulation.

Four patients received 16 mg/m^2, six received 20, seven received 25, and one received 30. One patient at 20 mg/m^2 died early. Hematologic toxicity for the remaining 17 patients is given in table 1. At 25 mg/m^2 the median wbc count nadir of 900/μl occurred at a median of 11 days. Severe leukopenia was seen at all doses but not in all patients at each dose. Recovery from nadir to > 3000/μl occurred at a median of 12 days. Platelet count nadir was less severe, but five courses were associated with a platelet count < 35,000/μl. At 25 mg/m^2 the median platelet count nadir was

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TABLE 1.—Hematologic toxicity

Dose (mg/m^2)	No. of— Evaluable patients	Evaluable courses	Median wbc count nadir (range)*	Median platelet count nadir (range)*
16	4	9	1.5 (0.8–3.4)	179 (45–305)
20	5	13	0.9 (0.7–1.7)	92 (20–161)
25	7	13	0.9 (0.4–1.2)	93 (18–151)
30	1	1	0.2	91

*$\times 10^6$ cells/μl.

93,000/μl, occurring at about 12 days. In the five courses with platelet count nadirs < 35,000/μl, recovery to 100,000/μl occurred 5–35 days later. Two patients at 16 mg/m^2, one at 20 mg/m^2, and two at 25 mg/m^2 received three courses; one patient at 20 mg/m^2 received four courses. The myelosuppression in successive courses was not more severe.

Four episodes of infection occurred. Two patients at 20 mg/m^2 developed pneumonia. The first patient had a wbc count of 900/μl when she became ill. The second patient had a wbc count of 1800/μl [absolute granulocyte count (AGC), 1170/μl], which occurred during recovery from surgery. Both patients recovered uneventfully, and the first received an additional course of high-dose MXT. A patient at 25 mg/m^2 developed gram-negative pneumonia requiring ventilatory support and a 22-day hospitalization. The admitting wbc count was 400/μl (AGC, 132), recovering to 2600/μl (AGC, 2153) in 6 days. After recovery she refused further MXT. The fourth episode occurred in the patient treated with 30 mg/m^2, who developed gram-negative sepsis at a wbc count of 200/μl. She was hospitalized for 12 days. Fourteen days later her wbc count was 2400/μl (AGC, 1248). She refused a second course.

There was no clinical evidence of cardiac deterioration during therapy. STIs were available following two or more courses from six patients; three showed significant deterioration (table 2). An additional patient had a significant decline in ejection fraction (68%–59%) following three courses of MXT. Additional toxic effects were nausea, vomiting, fatigue, and malaise (table 3). Three patients had mild alopecia. No hepatic or renal toxicity occurred. At 25 mg/m^2 moderate to severe fatigue occurred in six of 13 courses, detracting significantly from quality of life. Three patients refused second courses, two after infections.

DISCUSSION

Use of high-dose chemotherapy in solid tumors is supported by preclinical experimental evidence for a steep dose-response curve, even with non-cell-cycle-specific drugs and low-growth-fraction tumors (2,3). In the treatment of breast cancer, dose reduction has been implicated in reduced efficacy. A retrospective review of adjuvant chemotherapy suggested that benefit was dose-dependent (5). A review of data from several groups using cyclophosphamide, methotrexate, and 5-FU and cyclophosphamide, doxorubicin, and 5-FU for metastatic breast cancer related dose intensity (mg/m^2/week) to response as well as to median survival (4). Increasing the dose of drugs beyond that which produces "acceptable" myelosuppression deserves exploration in breast cancer but has been of limited benefit to date (9).

This trial shows that doses of MXT up to 30 mg/m^2 can be given every 3–4 weeks with relative safety. Moderate to severe myelosuppression occurred after nearly every course, but only four of 36 courses resulted in infection. Close monitoring of patients at the time of nadir and aggressive inpatient therapy for infection are required. The dose-limiting toxic effects in this popula-

TABLE 2.—Cardiac function

Dose (mg/m^2)	Patient No.	Dose (mg/m^2) Prior DOX	Prior MXT	Total MXT*	STI pretreatment	STI posttreatment
16	1	180	36	84	0.58	0.69†
	2	95	0	48	0.48	0.59†
20	3	390	0	40	0.53	0.46
	4	550	0	60	0.47	0.41
25	5	200	0	40	0.45	0.45
	6	218	24	74	0.44	0.51†

*Total prior to posttreatment STIs includes prior MXT and high-dose MXT.
†Significant increase.

TABLE 3.—Nonhematologic toxicity*

Toxic effect	Dose (mg/m^2)			
	16	20	25	30
Fatigue/malaise	1/9 (2)	8/13 (1.1)	9/13 (1.8)	1/1 (2.0)
Nausea/vomiting	4/9 (2.8)	2/13 (1)	3/13 (1.3)	0/1
Infection	0/9	2/13	1/13	1/1

*Values = No. of courses with toxicity/No. given (mean toxicity grade).

tion were fatigue and malaise. Indications of cardiotoxicity are difficult to interpret due to prior DOX and irradiation.

The single death was possibly drug-related. The patient had received prior DOX and chest irradiation but had normal cardiac function before receiving 20 mg/m^2 of MXT. The patient died 4 days later in her local hospital. No autopsy was performed. She did not have myelosuppression at the time of her death. Available information indicated that she died with a low cardiac output state. As a precaution, three additional patients at 20 mg/m^2 and two at 25 mg/m^2 were treated on an inpatient basis and 24-hour posttherapy gated radionuclide ejection fraction (MUGA) scans were obtained. None showed acute deterioration in cardiac function, so outpatient therapy was resumed.

Eighteen patients were treated with higher than usual doses of MXT. One indication of increased efficacy would have been responses in resistant patients; no such indication was seen in this trial. Determination of efficacy awaits phase II study in less heavily pretreated patients. The suggested phase II starting dose for this schedule is 25 mg/m^2 by iv push, repeated every 3-4 weeks as allowed by recovery.

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BRIEF REPORTS

Plasma Pharmacokinetics of 5-FU Given by Continuous Infusion With Allopurinol[1]

C. Erlichman,[2,*] S. Fine,[2] and T. Elhakim[3,4]

We determined the steady-state plasma levels of 5-FU when the drug was administered by continuous infusion for 5 days during 21 courses in patients with metastatic colorectal carcinoma receiving concomitant oral allopurinol given at a dose of 300 mg orally three times/day. The steady-state plasma level increased from 3.37 to 7.49 μM with dose increases from 1.25 to 2.25 g/m^2/day. Clearance ranged from 1.79 to 2.41 L/min/m^2. Although we observed a large day-to-day variation and a large patient-to-patient variation in plasma 5-FU levels at any given dose level, these were not significant. The plasma level of 5-FU increased linearly with dose. The difference between 5-FU plasma levels with dose was significant ($P = 0.002$). When the levels were adjusted for patient-to-patient and day-to-day variation the difference remained significant ($P = 0.02$). The results indicate that: (*a*) plasma levels of 5-FU may show large but insignificant variation from patient to patient and from day to day; and (*b*) steady-state plasma levels and 5-FU increased linearly with dose. [Cancer Treat Rep 70:903-904, 1986]

5-FU is a fluorinated pyrimidine which has been used clinically in the treatment of gastrointestinal, breast, and ovarian carcinomas since its introduction into clinical practice. In attempts to improve the therapeutic efficacy of 5-FU, studies which combine 5-FU with other nucleosides or compounds affecting pyrimidine metabolism have been explored (1-3). Whereas the pharmacokinetics of conventional 5-FU doses for iv bolus and continuous infusion therapies have been well documented (4-6), the combination of 5-FU with allopurinol allows the administration of higher doses of 5-FU than have been possible in the past (7-9).

We undertook to determine 5-FU steady-state concentration in patients receiving 5-FU by continuous infusion over 5 days in combination with allopurinol to define the dose dependence of 5-FU steady-state levels at doses which cannot be administered when the agent is given alone, to assess variation in 5-FU plasma concentrations from day to day and from patient to patient, and to determine whether nonlinear pharmacokinetics could be demonstrated under these treatment conditions.

MATERIALS AND METHODS

Plasma level monitoring of 5-FU was performed during 21 courses of treatment at various doses (table 1) for 5 days in patients receiving 300 mg of allopurinol orally, three times/day starting 1 day prior to the infusion and continuing for the 5 days of the 5-FU infusion. 5-FU was administered with an infusion pump in each of the monitored courses, with daily 5-FU doses divided into four equal parts and each portion delivered over a period of 6 hours in a solution of 5% dextrose in water which was protected from direct light. Each patient was sampled three to five times during the 5-day infusion. The initial samples were drawn 15-29 hours after the infusion began, and subsequent samples were drawn daily between 8 AM and 12 noon, at least 1 hour after the start of a 6-hour infusion and a dose of allopurinol. Plasma was analyzed for 5-FU using a previously published high-pressure liquid chromatography technique (10).

A least-square linear regression analysis of plasma concentration as a function of dose was performed to determine the relationship between these parameters. Analysis of variance was utilized to evaluate the significance of patient-to-patient variation and day-to-day variation at a given dose level and variation of plasma level at different doses. A similar analysis was repeated with total body clearance as the dependent variable. Total body clearance was determined using standard methods (11).

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TABLE 1.—Mean steady-state 5-FU plasma concentration and clearance at each dose level

Dose level (g/m^2/day)	1.25	1.5	1.75	2.0	2.25
No. of courses	4	2	4	7	4
No. of samples	15	6	14	25	17
C^{ss} ± SEM (μM)	3.37 ± 0.41	5.12 ± 0.96	6.35 ± 0.92	7.22 ± 0.71	7.49 ± 0.96
Clearance (L/min/m^2)	2.41	1.79	1.99	1.91	2.00

RESULTS AND DISCUSSION

This phase I study enabled us to determine the dose dependence of 5-FU plasma concentration and drug clearance in patients receiving the drug by continuous infusion with concomitant allopurinol. The number of courses monitored, the number of samples obtained, the plasma concentrations, and the total body clearance at each dose level are summarized in table 1. We found that 5-FU steady-state plasma level (C^{ss}) increased linearly with dose between the 1.25-g/m^2/day dose and the 2.25-g/m^2/day dose ($r = 0.43$, $P < 0.001$, t-test) and drug clearance did not change with increasing dose. The mean 5-FU plasma level increase with dose escalation was significant ($P = 0.002$). When patient-to-patient and day-to-day variation were included in the analysis as covariates, dose remained significant ($P = 0.02$). The use of infusion pumps, the aliquoting of the daily 5-FU dose into four 6-hour infusions which were protected from light, and the rapid separation of plasma followed by storage at −15°C until assay were measures used to minimize variable infusion rate, drug degradation in the infusion bag, and drug stability in plasma.

In a previous study, we had suggested that 5-FU demonstrated nonlinear pharmacokinetics (3). In this study, total body clearance did not change significantly with dose escalation and ranged from 1.79 to 2.41 L/min/m^2 (table 1) consistent with linear pharmacokinetics in the dose range studied. Howell et al (12) have described nonlinear pharmacokinetics when 5-FU and allopurinol were given in a manner similar to that reported here. The difference in results between our study and that of Howell may reflect patient-to-patient variation in the degradation of 5-FU by dihydrouracil dehydrogenase—a catabolic step which is saturable (13). The variability seen in the C^{ss} of 5-FU in this study and that of Howell makes the definition of nonlinear pharmacokinetics difficult unless a large number of patients can be studied at 5-FU doses > 2.0 g/m^2/day.

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Lhermitte's Sign as a Complication of Cisplatin-Containing Chemotherapy for Testicular Cancer[1]

R. Eeles, D. M. Tait, and M. J. Peckham[2,3,*]

Lhermitte's sign is reported in 14 patients receiving chemotherapy for metastatic testicular cancer. In six patients the typical symptoms appeared during chemotherapy: in five patients within 1 month of treatment and in one patient at 2 months. The duration of symptoms ranged from 2 to approximately 8 months. No patient had received radiotherapy to the cervical or dorsal spinal cord. The complication is ascribed to cisplatin, the only drug common to all 14 patients. [Cancer Treat Rep 70:905-907, 1986]

Lhermitte's sign (1), a sensation of electric shock or tingling of abrupt onset in legs and/or arms on neck flexion, is a well-recognized self-limiting complication of irradiation of the spinal cord (2). We have previously documented Lhermitte's sign in a patient receiving etoposide and cisplatin (3) and we here report on 14 patients who developed this complication after receiving combination chemotherapy for testicular cancer.

Clinical Observations

Between 1979 and 1985, 300 patients with testicular cancer received combination chemotherapy. Until the end of 1979, cisplatin, vinblastine, and bleomycin (PVB) (4) were employed. Subsequently, bleomycin, etoposide, and cisplatin (BEP) were used (5), and etoposide and cisplatin were used in selected patients (3).

Of the total group of patients, 14 were identified by direct questioning as having Lhermitte's sign. All patients described a sudden, momentary shock-like sensation involving the limbs. Five patients experienced shock sensations in the arms, and three in the hands, but the upper limbs were never the sole site. The feet were the most common site affected (eight patients); in two of eight patients no other site was reported. Eight patients' symptoms were provoked by sudden neck flexion. One patient was able to dissociate upper and lower limb sensation by different maneuvers. Two patients found that bending brought on shock sensations confined to the legs. One severely affected patient described a range of precipitating activities but also experienced Lhermitte's sign while lying still in bed or after taking alcohol.

Figure 1 summarizes the timing of onset of Lhermitte's sign in relation to chemotherapy and the duration of symptoms. Six patients developed symptoms on chemotherapy, four estimating the onset to be midway through treatment, and two describing it as appearing late in the course. Patient 3 experienced symptoms 2 months after chemotherapy. The remaining patients timed the onset to within the first month after chemotherapy.

The duration of symptoms was in the region of 1-3 months, but Patient 9 was affected for 8 months. Three patients (Nos. 2, 3, and 14) still have Lhermitte's sign after 6, 4, and 5 months, respectively.

Every patient experienced one or more other symptoms attributable to drug toxicity. The strongest association was with peripheral neuropathy (12 patients), the onset of which coincided with Lhermitte's sign in all but one patient. Raynaud's phenomenon was also commonly seen (nine patients), but the timing of onset was more varied. Apart from Patient 13, whose Raynaud's phenomenon resolved after 2 months, peripheral neuropathy and Raynaud's phenomenon are ongoing symptoms in all affected patients, with a duration varying from 3 to 54 months (table 1).

The only drug common to all patients is cisplatin. There is no obvious correlation between the dose of cisplatin and the risk of developing Lhermitte's sign. All patients had normal renal function prior to chemotherapy and none experienced severe renal impairment as a result of treatment. None of the Lhermitte's sign patients had dorsal or cervical spine irradiation, although three patients received abdominal node irradiation. However, in these cases, 6-8 months elapsed between

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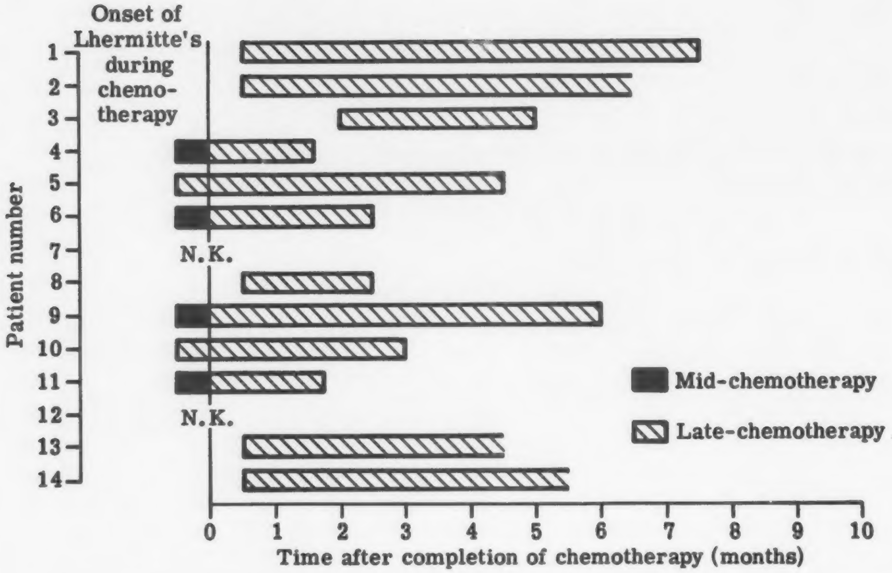

FIGURE 1.—Time of onset and duration of Lhermitte's sign in relation to chemotherapy. N. K. = not known.

irradiation and chemotherapy with no evidence of Lhermitte's sign during this time.

Since cisplatin was the only drug common to all patients in the present series, Lhermitte's sign is presumed to represent a complication of this drug, the neurotoxicity of which is well recognized (6-12).

A platinum dose-effect for Lhermitte's sign has not been demonstrated in this group of patients. It is of interest that some patients were aware of Lhermitte's sign after only two to three courses of cisplatin, when a cumulative dose of only 360-540 mg had been given. Other patients had received a far higher dose (3000 mg) before becoming symptomatic. This suggests a wide variation in sensitivity. The appearance of symptoms of peripheral neuropathy in parallel with Lhermitte's sign in most of the patients might suggest a similar causal mechanism. In all patients with both Lhermitte's sign and peripheral neuropathy, however, the latter is of longer duration than the former. Evidence of a platinum dose-effect for peripheral neuropathy has been described with different cumulative doses for signs and symptoms (12).

Lhermitte's sign is a further example of platinum neurotoxicity. Patients who experience this complication can be reassured that it is not likely to result in long-term morbidity.

TABLE 1.—Association of Lhermitte's sign with peripheral neuropathy and Raynaud's phenomenon

Patient No.	Peripheral neuropathy (duration in mos)	Raynaud's phenomenon (duration in mos)
1	+ (5–6)	+ (28)
2	+ (20)*	+ (6)*
3	— —	+ (12)*
4	+ (42)*	+ (54)*
5	+ (20)*	+ (18)*
6	+ ( )*	— —
7	+ ( )*	+ (24)*
8	+ (3)*	— —
9	+ (10)*	+ (10)*
10	+ (9)*	— —
11	+ (3)*	+ (4)*
12	N.K.† —	N.K.† —
13	+ (7)*	+ (2)
14	+ (5)	— —

*Symptoms persisting.
†N.K. = not known.

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BRIEF REPORTS

Phase II Study of Cisplatin, 5-FU, and Allopurinol in Advanced Esophageal Cancer[1]

Pietro De Besi,* Vanna Chiarion Sileni, Luigi Salvagno, Carlo Tremolada, Giuseppe Cartei, Vinicio Fosser, Adriano Paccagnella, Alberto Peracchia, and Mario Fiorentino[2]

Forty patients with advanced squamous cell carcinoma of the esophagus were treated with a combination of cisplatin, 5-FU (by continuous 5-day infusion), and allopurinol; 37 are evaluable for response. Thirteen remissions (35%) were obtained, including three complete and ten partial, with a median duration of 9 months. After chemotherapy, seven responding patients underwent a surgical procedure, which was radical in four. The most frequent side effects were nausea and vomiting. This regimen is effective and can be included in a multimodality approach. [Cancer Treat Rep 70:909-910, 1986]

The combination of cisplatin and 5-FU, widely applied in squamous cell carcinoma of the head and neck (1), was also used in a limited group of patients with esophageal cancer (2). With a phase II study, we have tried to further clarify the response rate and the toxicity of this combination in esophageal cancer. Allopurinol was added to reduce bone marrow and gastrointestinal toxicity (3,4).

MATERIALS AND METHODS

Forty patients with advanced, histologically proven squamous cell carcinoma of the esophagus were entered. Ten patients had undergone previous operations and were relapsing. The patients' main characteristics are described in table 1.

All of the patients had complete medical history, blood chemistry, chest x-ray, barium esophagram, esophagoscopy, tracheobronchoscopy, abdominal ultrasound or computerized tomography, and, when indicated, laparoscopy. These examinations were done initially and repeated at every two cycles. Patient entry required adequate laboratory parameters (wbc count $\geq 4000/\mu l$, platelet count $\geq 100{,}000/\mu l$, BUN < 40 mg/dl, and creatinine < 1.5 mg/dl) and Karnofsky performance status > 40.

The treatment schedule consisted of allopurinol at a dose of 600 mg/day orally from Days -2 to $+5$, cisplatin at a dose of 100 mg/m^2 iv on Day 1 in a 2-hour infusion (5), and 5-FU at a dose of 1000 mg/m^2/day as a continuous 5-day infusion. The course was repeated on Day 22. Dose escalation was not planned. In case of leukopenia (< 4000 cells/μl) or thrombocytopenia ($< 100{,}000$ cells/μl), the course was delayed to permit hematological recovery. Responders received a maximum of six courses, whereas in patients with disease progression, the treatment was discontinued. No nutritional support was administered during the treatment program.

Response criteria were as follows: *complete response*—the total disappearance of the tumor both radiologically and histologically for a minimum of 1 month; and *partial response*—a $> 50\%$ decrease in the volume of every measurable lesion. Response duration was calculated from the start of therapy. Toxicity was reported according to the World Health Organization scale.

RESULTS

Of the 40 patients entered into this study, three were not evaluable for response: two refused further therapy after the first course and one died within 36 hours after the beginning of the chemotherapy.

The median number of courses per patient was four (range, two to six). Among the 37 evaluable patients, there were 13 objective responses (35%), including three complete and ten partial. Among the responders, seven patients (one complete and six partial responses) with previously unresectable locoregional disease underwent surgical exploration after two to four courses. Four radical and two palliative resections were carried out, whereas in a seventh patient, despite computerized tomographic scan findings, the primary was so densely adherent to the trachea that surgical resection was not possible. Of the six resected patients, the two with palliative and one of those with radical resection died of progressive intrathoracic disease after 2, 5, and 13 months,

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TABLE 1.—Main characteristics of the patients

Characteristic	No. of patients
Entered/evaluable	40/37
Males/females	37/3
Median age in yrs (range): 59 (37–70)	
Median Karnofsky performance status (range): 70 (50–90)	
Extent of disease	
Locally advanced tumor	21
Primary tumor and distant metastases	11
Distant spread (primary removed)	4
Anastomotic recurrence	4
Previous treatment	
Surgery	10
Radiotherapy	4
Chemotherapy	1
None	25

respectively. The other three radically resected patients are now alive with no clinical evidence of disease at 6, 13, and 26 months, respectively.

The overall median duration of response was 9 months (range, 2–26+). This chemotherapy regimen was well tolerated: nausea and vomiting were common after cisplatin infusion, and reversible renal damage (grade 1), which caused a delay in the administration of the subsequent course, was seen in one patient. Leukopenia (grade 1 and 2) and thrombocytopenia (grade 2), evaluated on the day of recycling, were observed in ten and one patient, respectively; a delay of 7 days permitted recovery and full dose recycling. Mucositis (grades 2 and 3) occurred in five patients and caused a delay of 7 days in four. Liver enzyme changes (gamma GT, transaminases) were observed soon after 5-FU infusion in 12 patients; in ten patients they returned to the normal range within 10 days, while in two a delay of 8 days was necessary. There was one unexpected early death 36 hours after the beginning of the chemotherapy, and a previously undetected diffuse angiosclerosis was found at autopsy.

DISCUSSION

When used as single agents, cisplatin and 5-FU produced response rates of 22% and 15%, respectively, with a median duration of response not exceeding 3 months (6). When the two drugs were used in combination, the response rate was higher (80%), suggesting that cisplatin can act synergistically with 5-FU (2). In our study, we added allopurinol in order to reduce bone marrow and gastrointestinal toxicity as indicated by experimental and clinical experiences (3,4).

Our results suggest that a relatively high response rate (35%) can be reached even in the presence of advanced tumor, but they do not confirm the previous report by Hellerstein et al (2). In their study, the number of patients was low (ten) and there was high prevalence of limited tumors, which may explain the discrepancy with our results.

This treatment was generally well tolerated and side effects were mild and promptly reversible. It is noteworthy that as a consequence of this chemotherapy, seven nonoperable cases were judged as probably operable, and a radical resection was possible in four. Such results make it advisable to perform further studies to determine how best to integrate this or similar systemic treatment with surgery and radiation in the early phase of the disease in order to improve the long-term therapeutic results.

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CLINICAL TRIALS SUMMARIES

Phase II Evaluation of Oral Idarubicin (4-Demethoxydaunorubicin) in Patients With Disseminated Malignant Melanoma[1]

**Massimo Lopez,[2,*] Luigi Di Lauro,[2] Paola Papaldo,[2] Fabrizio Ganzina,[3]
Nicola Di Pietro,[3] and Bonaventura Lazzaro[2]**

Idarubicin (4-demethoxydaunorubicin) is an analogue of daunorubicin lacking the methoxyl group in the 4 position of the aglycone. The compound was found to be active in a variety of experimental murine tumors by both oral and parenteral routes of administration (1,2). Moreover, it was significantly more potent than daunorubicin and doxorubicin with a better therapeutic index in comparison to the earlier drugs, particularly as regards cardiotoxicity (1). Phase I studies suggested a starting dose of 40 mg/m^2 orally every 3-4 weeks, with an increase to 50 mg/m^2 in the absence of significant myelotoxicity (3). Responses in melanoma patients were seen. Hence, a phase II study of oral idarubicin in patients with disseminated malignant melanoma was initiated in the hope that this new drug would be found active in this disease.

PATIENTS AND METHODS

Twenty-six patients with biopsy-proven disseminated malignant melanoma and measurable disease were entered in the study. One patient was considered unevaluable because of loss to follow-up after the first cycle of therapy. Principal characteristics of the 25 fully evaluable patients are summarized in table 1. Idarubicin was administered in single oral doses of 45 mg/m^2 in good-risk patients, and of 40 mg/m^2 in poor-risk patients, with cycles repeated at intervals of 3-4 weeks. Due to severe toxicity encountered in a previous study in patients with breast cancer given doses of 50 mg/m^2, no attempts were made at dose escalation. Treatment was continued as long as the disease was stable or responding, with no excessive toxic effects. Response and toxicity were evaluated according to World Health Organization criteria (4).

RESULTS

There were only two (8%) responses, both in patients without prior chemotherapy and with nonvisceral-dominant disease. One of these responses was complete and occurred in a patient with nodal and subcutaneous nodules (time to progression, 232 days). Disease stabilization (60 days) was observed in a third patient with subcutaneous nodules. The probability of having a true response rate $\geq$ 20% is only 9.2%.

Toxic effects were primarily hematologic. Leukopenia was observed in 70% of the courses. The median wbc count nadir was 2900/mm^3 (range, 800-9800) and was reached on Day 13 (range, 5-22). Full recovery occurred in 63% and 91% of the courses by Days 21 and 28, respectively. Thrombocytopenia was encountered in only 8% of the courses. The median platelet count nadir was 180,000/mm^3 (range, 25,000-300,000), with full recovery in 93% of the courses by Day 21. Nausea and vomiting were generally mild to moderate. No patients developed any sign or symptom of cardiotoxicity.

DISCUSSION

This phase II trial of oral idarubicin in patients with metastatic malignant melanoma appears to have demonstrated that the drug does not have significant antitumor activity at a dose level that produced definite but moderate and acceptable hematologic toxicity. Nausea and vomiting were generally so mild and short-lived that an antiemetic therapy was not required. Nevertheless, mild vomiting encountered in 55% of the courses could have reduced the bioavailability of the drug, possibly inadequately dosing the nonresponding patients. It

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should be noted, however, that vomiting started generally 3-4 hours after drug administration, and leukopenia was recorded in most patients who experienced vomiting.

TABLE 1.—Patient characteristics and treatment results*

Characteristic	
Total evaluable	25
Median age in yrs (range)	54 (37-75)
Median ECOG performance status (range)†	1 (0-3)
Metastases	
Nonvisceral	14
Visceral	11
Prior therapy	
Surgery	25
Chemotherapy	12
Radiotherapy	6
Immunotherapy	6
Response	
Complete	1
Partial (> 50%)	1
Stable disease	1
Progressive disease	22
Toxicity‡	
Nausea and vomiting	
Grade 1	28
Grade 2	55
Stomatitis	
Grade 2	2
Diarrhea	
Grade 1	9
Grade 2	5
Grade 3	5
Fever	
Grade 1	4
Alopecia (% of patients)	
Grade 1	4
Leukopenia	
Grade 1	16
Grade 2	39
Grade 3	11
Grade 4	4
Thrombocytopenia	
Grade 1	4
Grade 2	2
Grade 3	2

*Idarubicin dose: 40-45 mg/m^2 orally every 3-4 wks. Unless otherwise specified, values = No. of patients.

†ECOG = Eastern Cooperative Oncology Group.

‡% of 58 courses; grading according to Miller et al (4).

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CLINICAL TRIALS SUMMARIES

Guanazole in the Treatment of Advanced Bronchogenic Carcinoma: A Pilot Study of the Southeastern Cancer Study Group[1,2]

Stephen Krauss,[3,*] Lawrence E. Broder,[4] and Robert Birch[5]

Guanazole, whose mechanism of action is similar to that of hydroxyurea, showed some activity against lung cancer in early phase I trials (two of two partial responses) (1). We report our phase II experience with this agent.

Patients were eligible for the study if they had microscopically proven unresectable bronchogenic carcinoma of any cell type. Patients could have received prior surgery, radiation therapy, or chemotherapy (except cytarabine or hydroxyurea). A Karnofsky performance status ≥ 60%; normal hematopoietic, renal, and hepatic functions; at least one measurable or evaluable tumor lesion; and written informed consent were required.

Guanazole was given iv at a dose of 4 g/m^2 over 1 hour every 8 hours for 5 days, repeated every 3 weeks for at least two cycles (1). Patients were evaluated for response and toxicity using Southeastern Cancer Study Group criteria (2,3). Responders continued on the drug until disease progression.

Patient characteristics and treatment results are shown in table 1.

Twenty-two patients were entered in the study; three were evaluable for toxicity only. There was one partial response in a patient with large cell carcinoma previously treated with chemotherapy and radiation therapy.

Severe to life-threatening hematologic toxicity (grades 3-4), ie, granulocytopenia, occurred in 11 patients, of whom nine had received extensive prior therapy. Other toxic effects included mild to moderate elevation in hepatic enzymes (six patients), nausea (three), and decrease in performance status (17), which was pronounced in nine. Nine patients, including the single responder, who continued guanazole beyond two cycles subsequently showed evidence of disease progression. All patients have died, with a median survival of 9 weeks; the longest survivor lived 56 weeks.

TABLE 1.—Patient and treatment characteristics: phase II pilot study of guanazole*

Characteristic	No. of patients (%)
Entered	22
Evaluable†	19
Male	16
Female	6
Median age in yrs (range): 60 (40–75)	
Histology	
Adenocarcinoma	11
Squamous cell carcinoma	6
Large cell carcinoma	4
Small cell carcinoma	1
Prior therapy	
None	6
Minimal	
Irradiation alone	6
Surgery alone	2
Extensive	
Chemotherapy and irradiation	9
Karnofsky performance status ≥ 70%	20
Response	
Complete	0
Partial	1
Stable disease	9
Disease progression	6
Death	3
Toxicity	
Mild	1 (5)
Moderate	3 (14)
Severe (granulocytopenia)	6 (27)
Life-threatening (granulocytopenia)	11 (50)

*Drug given as follows: 4 g/m^2 iv q 8 hrs × 5 days, repeated q 21 days for at least 2 cycles.

†3 patients evaluable for toxicity only.

In this study of patients with advanced lung cancer (most of whom were pretreated), guanazole produced a

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partial response in only one of 22 patients. Further studies with this agent as given in this study in patients with lung cancer do not appear warranted.

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Treatment of Advanced Adenocarcinoma of the Kidney With ICRF-187: A Southeastern Cancer Study Group Trial[1,2]

Leonard H. Brubaker,[3] Charles L. Vogel,[4] Lawrence H. Einhorn,[5] and Robert Birch[6,*]

The Southeastern Cancer Study Group (SEG) has completed a phase II trial in patients with renal adenocarcinoma using ICRF-187, the D-enantiomer of razoxane, a drug with anticancer activity but variable bioavailability (1).

Eligibility for this trial was the same as on a previous SEG trial (2). Signed informed consent was required. Patients with bilirubin < 1.2 mg/dl and no prior radiation therapy were treated with a course of ICRF-187, 3.8 g/m^2/week iv for four doses, followed by a 2-week rest, followed by a second course with a 25% increase in dose if there had been no grade 2 neutrophil toxicity. If patients had had prior radiation therapy to marrow-bearing areas and/or prior chemotherapy, the initial ICRF-187 dose was 2.6 g/m^2/week for the first course with a 25% escalation for the second course. Subsequent to the second course, there was no further dose escalation. Criteria for response and toxic effects were according to SEG standards (3).

Of 40 patients entered, nine had doses escalated; others were not escalated because of the short duration of treatment before progression, toxicity in the first course, or (six cases) the investigator not escalating as requested. Five patients had fewer than three doses. Five patients received the 2.6-g/m^2 starting dose as per protocol. The great majority of patients received the maximum doses of ICRF-187 permitted by the protocol. There were no antitumor responses seen in the 40 patients. More significantly, there were no responses among the 16 patients evaluable for response who had biological evidence of adequate dose as manifested by grade 2 or greater neutrophil and/or liver (SGOT) toxicity.

Patient characteristics and drug toxicity are summarized in tables 1 and 2. Blood count depression and transient SGOT elevation were generally rapidly reversible. There was no evidence of dose-related or cumulative toxicity. Also, liver toxicity, as measured by increased SGOT, was unrelated to pretreatment SGOT. Prior myelosuppressive therapy did not increase the chance of any grade 2 toxicity. There was one death associated with grade 2 neutrophil toxicity and infection. Two patients developed altered mental status (confusion and depression) that may have been related to the drug.

TABLE 1.—Patient characteristics

Total No. of patients	40
Males	85%
Females	15%
Age in yrs	
Median	56
Range	24–71
Initial performance status	
Median	80
Range	50–100
No. (%) of patients with—	
No prior chemotherapy or hormones	23 (58%)
Minimal prior treatment (1 agent)	10 (25%)
Extensive prior treatment	7 (17%)
No. (%) of patients with sites of metastasis or spread to—	
Lung	29 (73%)
Bone	12 (30%)
Liver	7 (18%)
Abdomen	11 (28%)
Supraclavicular nodes	2 (5%)
Brain	6 (15%)
History (wks)*	
Median	17
Range	1–76
No. of doses received/patient†	
Mean ± 1 SD	7 ± 5.8
Range	1–28
Results	
Responders	0

*No. of wks from symptoms or diagnosis of recurrence to entry.
†4 doses = 1 course.

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TABLE 2.—Maximum toxicity

Toxic effect	% of patients
Neutrophil count, grade 2-3	25
Neutrophil count, grade 4	3
Platelet count, grade 2	3
SGOT, grade 2-3 (2-10 × normal)	35
Bilirubin, grade 1	3
Nausea, grade 1	26
Nausea and minimal vomiting, grade 2	12
Stomatitis, grade 1-2	14
Diarrhea, grade 1-2	17
Hair loss, grade 1-2	33
Phlebitis, grade 3 (no other reported)	3
Allergy, rash	6

In the dose and schedule used, ICRF-187 had no detectable activity against renal adenocarcinoma. Toxicity was similar to that previously reported (4).

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CLINICAL TRIALS SUMMARIES

Phase II Eastern Cooperative Oncology Group Study of Spirogermanium in Previously Treated Lymphoma[1,2]

László Boros,* Anastasios A. Tsiatis, Richard S. Neiman, Risa B. Mann, and John H. Glick[2,3]

Spirogermanium is an azaspirane that incorporates the element germanium into the heterocyclic ring structure. The mode of action appears to be through inhibition of protein synthesis, with secondary depression of RNA and DNA synthesis (1). The Eastern Cooperative Oncology Group (ECOG) conducted this phase II study of spirogermanium in previously treated patients with lymphoma because of the prior reported antilymphoma activity of this drug (2–4).

PATIENTS AND METHODS

All patients had histologic proof of malignant lymphoma (either low or high grade) or Hodgkin's disease. Eligibility criteria included measurable stage III or IV disease; at least one, but no more than two, prior chemotherapy trials with failure to respond or relapse from complete or partial remission; adequate hematologic reserve; and good performance status (ECOG criteria). Patient characteristics are shown in table 1.

Spirogermanium was given at a dose of 80 mg/m^2 iv in 150 ml of saline over 60–90 minutes every other day three times per week for 2 weeks. If tolerated at that level, the dose was escalated to 120 mg/m^2 as a single dose weekly. Fourteen patients had dose escalation, with no increase in toxicity. Therapy was continued until there was evidence of disease progression.

RESULTS

Thirty-three patients were entered in this trial, with 25 evaluable for response. Treatment was discontinued in two patients, two were inevaluable for both toxicity and response, and four were inevaluable for response. Two partial responses were seen, one lasting 90 days (diffuse large cell lymphoma) and the other ongoing at 7 months (diffuse poorly differentiated lymphocytic lymphoma). Both of these patients had prior complete response to chemotherapy. Two patients had stable disease, one lasting 4 months and the other lasting 8 months. Median time to disease progression was 19 days (range, 4–118). Twenty-three patients have died. Median survival from date of entry in the study was 5 months (range, 6 days to 3 years).

Toxicity was generally moderate, being less than grade 2 for all systems, except in three patients who had seizures. One patient died of seizures the day after a dose of 80 mg/m^2 while febrile to 102°F. Another patient, with a prior history of seizures 3 years before, had a grand mal seizure 1½ hours after the first dose escalation to 120 mg/m^2. A third patient had seizures 15 days after a dose of 120 mg/m^2. An additional patient had pneumonia and sepsis with progressive lethargy and somnolence, leading to death 3 days following the third dose of 80 mg/m^2. Other central nervous system toxic effects included tinnitis or paresthesia (six patients); light-headedness (four); lethargy (two); and hyperactivity, blurred vision, insomnia, somnolence, vertigo, and headache (one each).

DISCUSSION

Spirogermanium has very little activity in pretreated, relapsed patients with lymphoma. Based on this lack of activity, the ECOG will not advance this treatment to

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[2] This study was conducted by the Eastern Cooperative Oncology Group (*Paul P. Carbone*, chairman, CA-21115) and was supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. The following members participated in the study: University of Rochester Cancer Center, NY (*L. Boros*, CA-11083); Dana-Farber Cancer Institute, Boston, MA (*A. A. Tsiatis*, CA-23318); Mallory Institute of Pathology, Boston University Medical Center, MA (*R. S. Neiman*, CA-21115); Johns Hopkins Oncology Center, Baltimore, MD (*R. B. Mann*, CA-16116); and Hospital of the University of Pennsylvania, Philadelphia (*J. H. Glick*, CA-15488). Other participating institutions include: Albany Medical College, NY (CA-06594); American Oncologic Hospital, Philadelphia, PA (CA-18281); Brown University and Roger Williams General Hospital, Providence, RI (CA-15947); Mayo Clinic, Rochester, MN (CA-13650); Medical College of Ohio, Toledo; University of Minnesota, Minneapolis (CA-20365); New York University Medical Center, New York, NY (CA-16395); Newark Beth Israel Medical Center, Newark, NJ; Northwestern University Medical Center, Chicago, IL (CA-17145); University of Ottawa, Ontario, Canada; Pennsylvania Hospital, Philadelphia (CA-13613); University of Pittsburgh, PA (CA-18653); Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL (CA-25988); University of Pretoria, South Africa (CA-21692); Natalie Warren Bryant Cancer Center, Tulsa, OK; Wisconsin Clinical Cancer Center, Madison (CA-21076); and Yale School of Medicine, New Haven, CT (CA-15485).

[3] We thank Mrs. Sheila Hutchings for her expert help in preparing this manuscript.

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higher-phase studies in lymphomas. The most important contribution of this study was to emphasize that patients with previous seizure history should not be treated with spirogermanium.

TABLE 1.—Patient characteristics

Characteristic	No. of patients ($n = 31$)
Median age in yrs (range): 62 (24–76)	
Performance status*	
0–1	21
2	10
Prior chemotherapy	
1	7
2	17
>2	7
Prior radiotherapy	19
Stage	
I	1
III	6
IV	24
Histology	
Hodgkin's disease	3
Diffuse histiocytic lymphoma	16
Diffuse poorly differentiated lymphoma	6
Nodular lymphoma	6
Response	
Partial	2†
Stable disease	2‡

*ECOG scale.
†3 and 7+ mos.
‡4 and 8 mos.

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CLINICAL TRIALS SUMMARIES

Ifosfamide Plus *N*-Acetylcysteine in the Treatment of Small Cell and Non-Small Cell Carcinoma of the Lung: A Southeastern Cancer Study Group Trial[1,2,3]

Patrick J. Loehrer, Sr,[4,*] Robert Birch,[5] Barnett S. Kramer,[6] F. Anthony Greco,[7] and Lawrence H. Einhorn[4]

Ifosfamide (IFX) is an oxazphosphorine which has been reported to have a 30%-35% response rate in bronchogenic carcinoma (1,2). More recent studies, however, by Slavik and Saiers (3) and the Southwest Oncology Group (R. Livingston, personal communication) have questioned this degree of activity. We, therefore, initiated a phase II trial to confirm the activity of IFX in patients with advanced bronchogenic carcinoma.

PATIENTS AND METHODS

From January 1983 to February 1984, 102 patients were entered in the study. Patient characteristics are shown in table 1. All patients had bidimensionally measurable or evaluable disease, performance status > 60, serum creatinine ≤ 2 mg/dl, and platelet and granulocyte counts $> 100{,}000$ and $200{,}000/\text{mm}^3$, respectively. All patients with small cell carcinoma had at least one prior chemotherapeutic regimen (12 of these latter patients had at least two prior regimens). IFX (1.2 g/m^2) was administered daily for 5 consecutive days. *N*-Acetylcysteine capsules (2.0 g) were administered every 6 hours during treatment days until 36 hours after the last dose of IFX as a urothelial protective agent. Continuous hydration with 0.45% normal saline was given at a rate of 100 ml/hour. Standard criteria for response were used.

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Eligible	100
Evaluable	84
Median age in yrs (range): 60 (40–85)	
Male:female	80:20
Median Karnofsky performance score (range): 80 (60–100)	
Prior chemotherapy	31
Prior radiation therapy	50
Sites of metastasis	
Bone	34
Liver	28
Lymph node	21
Pleura	20
Brain	13
Skin	10
Bone marrow	4
Histology	
Squamous cell carcinoma	42
Adenocarcinoma	27
Large cell carcinoma/undifferentiated carcinoma	8
Small cell carcinoma	23
Responses ($N = 6$)*	
No prior therapy	
Squamous cell carcinoma ($N = 24$)	0
Adenocarcinoma ($N = 13$)	1†
Large cell carcinoma ($N = 4$)	1‡
Prior therapy	
Squamous cell carcinoma ($N = 13$)	2†
Adenocarcinoma ($N = 10$)	1†
Large cell carcinoma ($N = 2$)	0
Small cell carcinoma ($N = 18$)	1†

*Median survival for patients with non-small cell carcinoma, 23.6 wks; and for patients with small cell carcinoma, 10.2 wks.

†Partial response.

‡Complete response.

RESULTS

Among the 84 patients eligible for response [two were ineligible; eight were evaluable for toxicity only; five had early death (non-drug-related); two refused therapy; and one had inadequate data], there were five partial responses and one complete response (combined response rate, 7%; 95% confidence limits, 3%-15%). Two of 41 patients without prior chemotherapy (95% confidence limits, 1%-18%) and four of 43 with prior chemo-

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[3] Ifosfamide and *N*-acetylcysteine were supplied by Mead-Johnson Pharmaceutical Division, Evansville, IN.

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TABLE 2.—Toxic effects

Toxic effect	No. of patients
Hematologic (cells/mm^3)	
Granulocyte count nadir	
1000–1499	11
500–999	5
< 500	9
Platelet count nadir	
90,000–130,000	4
50,000–89,999	6
25,000–49,999	2
$< 25,000$	0
Renal	
BUN (mg/dl)	
21–40	10
41–60	2
Symptomatic uremia	1
Serum creatinine (g/dl)	
1.3–2.0	11
2.1–3.0	1
> 3.0	1
Hematuria	
Microscopic	13
Gross hematuria	10
Nausea/vomiting	
Mild	18
Moderate	35
Severe	5
Central nervous system disturbance	24*
Peripheral neuropathy	4
Pulmonary symptoms	16

*Includes mood changes or confusion (12 episodes), headaches (3), cerebellar dysfunction (5), motor dysfunction (1), state of consciousness (12), and ideation (10).

therapy (95% confidence limits, 3%–23%) responded. Only one of 18 patients (95% confidence limits, 0%–29%) with refractory small cell carcinoma responded to IFX. The median survival times for evaluable patients with non-small and small cell carcinoma are noted in table 1.

Toxicity was primarily gastrointestinal, including 33% of the patients with moderate to severe weight loss (table 2). Central nervous system toxicity occurred in 24% of the patients. Clinically, we felt that these toxic effects represented an adequate dosage administration to evaluate the efficacy of IFX.

DISCUSSION

Although results from earlier trials with both cyclophosphamide and IFX in bronchogenic carcinoma have demonstrated response rates in $> 20\%$ of the patients, more recent investigations with cyclophosphamide have questioned this degree of activity (4). This current trial fails to demonstrate a high degree of activity for IFX in non-small cell or refractory small cell carcinoma, even when excluding patients with early deaths. The number of early deaths with brain metastases and lack of response in extensively pretreated patients with small cell cancer underscores the current National Cancer Institute recommendations to exclude such patients with a markedly unfavorable prognosis. We feel that further trials with IFX in bronchogenic carcinoma are not warranted.

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CLINICAL TRIALS SUMMARIES

Phase II Evaluation of Mitoxantrone in Advanced Renal Cell Carcinoma: A Southeastern Cancer Study Group Trial[1,2]

Richard A. Gams,[3] Owens Nelson,[4] and Robert Birch[5,*]

Mitoxantrone is a member of a group of bis-substituted anthraquinones with antineoplastic activity (1). In preclinical studies, the drug was shown to be active against iv implanted L1210 leukemia and a subline of P388 leukemia resistant to doxorubicin (2). As part of a series of phase II trials of mitoxantrone, we evaluated this drug in patients with advanced renal cell carcinoma using a weekly dose schedule. The recommended starting dose was 5 mg/m^2 (3).

To be eligible for this study, patients must have had a diagnosis of metastatic renal cell carcinoma with measurable lesions, a Karnofsky performance status of $\geq 40\%$, bilirubin < 1.2 mg/100 ml, and a granulocyte count $>$ 2000/mm^3. All patients must have given informed consent and may or may not have had prior therapy. Mitoxantrone was given iv over a 30-minute infusion on a schedule of 5 mg/m^2/week. Doses were not escalated but were reduced by 25% if the granulocyte count fell below 1400/mm^3 or the platelet count fell below 90,000/mm^3.

Patients were evaluated for response after 6 weeks of therapy. A partial response was defined as a 50% decrease in the sum of the products of the diameters of all measurable lesions, which persisted for at least 4 weeks.

Fifty-eight patients were entered on this study (table 1), of whom 48 (84%) were considered evaluable for response. One patient was not eligible, seven patients did not have enough data recorded to permit evaluation of response, and two were lost to follow-up.

No complete or partial responses were seen. The treatment was well tolerated and the predominant side effect was granulocytopenia. Of the 50 patients evaluable for toxic effects, 41 (82%) experienced absolute granulocyte counts < 1900/mm^3 and 31 (62%) experienced absolute granulocyte counts < 1000/mm^3. The blood cell counts returned to normal with cessation of therapy. Thrombocytopenia occurred infrequently and was generally mild. Nausea and vomiting was reported in 19

TABLE 1.—Patient characteristics

Characteristic	No. of patients (%)
Prior radiotherapy	
Yes	20 (34)
No	38 (66)
Prior chemotherapy	
Yes	27 (47)
No	31 (53)
Performance status	
40	2 (3)
50	1 (2)
60	13 (22)
70	16 (28)
80	15 (26)
85	1 (2)
90	7 (12)
100	3 (5)
Sex	
Male	43 (74)
Female	15 (26)
Age	
≤ 50	17 (29)
51-60	23 (40)
≥ 61	18 (31)
Lung/pleural metastases	
Unknown	2 (4)
Yes	43 (74)
No	13 (22)
Liver metastases	
Unknown	7 (12)
Yes	6 (10)
No	45 (78)
Brain metastases	
Unknown	9 (16)
Yes	2 (3)
No	47 (81)
Bone metastases	
Unknown	7 (12)
No	32 (55)
Yes	19 (33)

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patients (38%) but was generally mild so as not to require antiemetics. A variety of other adverse effects including fever, stomatitis, diarrhea, ECG changes, and weight loss occurred sporadically and were generally mild.

Mitoxantrone, when given in this dose and schedule, is well tolerated but produced no responses in advanced renal cell carcinoma. This result is similar to that observed by the Southwest Oncology Group using a dosage of 14 mg/m^2 given every 3 weeks (4). In retrospect, more patients were accrued to the study (especially in the category of no prior chemotherapy) than should have been necessary to establish the lack of activity of this dose schedule; this reflects the importance of having early stopping rules for clinical trials. We now employ a two-stage sampling scheme in such studies and would suspend the trial after the first 14 patients in each treatment category to determine the presence or absence of responses. We conclude that mitoxantrone has no role in the management of renal cell carcinoma.

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Tamoxifen in the Treatment of Refractory Adenocarcinoma of the Cervix[1]

John J. Kavanagh,[2,*] J. Taylor Wharton,[3] and Felix Rutledge[3]

Tamoxifen is a nonsteroidal antiestrogen. The compound is an analog of clomiphene with weakly estrogenic activity (1). In dimethylbenzanthracene-induced mammary cancer, tamoxifen is able to inhibit already established or new tumor growth (2). The mechanism of action is thought to be a blockade of estrogen receptors in the cytosol. This may result in an occupied receptor interfering with messenger RNA synthesis within the nucleus (3). Tamoxifen is commonly used for the treatment of metastatic breast cancer. However, responses have also been noted in endometrial adenocarcinoma (4). In patients with refractory ovarian adenocarcinoma, the drug produces stability of disease with minimal responses (5). For these reasons, a trial of tamoxifen in refractory adenocarcinoma of the cervix was undertaken.

MATERIALS AND METHODS

Fifteen patients who had failed primary surgical or radiotherapy treatment received tamoxifen. All patients had glandular adenocarcinoma. Those patients with squamous elements or histologic variants as clear or glassy cell carcinoma were excluded. The tamoxifen dose was 20 mg orally twice a day, except for one patient who received 10 mg orally twice a day. A patient was considered evaluable if she received 6 weeks of therapy. Estrogen and progesterone receptors were not determined on the tumors.

One patient had been treated with a progestational agent for the malignancy. One patient each had had exposure to estrogens within the 2 years prior to receiving tamoxifen. No patient concurrently received any steroid medication while receiving the antiestrogen. All patients were considered postmenopausal based on spontaneous cessation of menses or the consequences of surgery and radiation therapy. Patient characteristics are described in table 1.

All patients had received prior chemotherapy. Eleven patients (73%) had been treated with the combination of 5-FU, doxorubicin, and cisplatin. Two other patients had been given cisplatin and cyclophosphamide. Nine patients (60%) had completed a trial of 5-day continuous-infusion vinblastine.

RESULTS

Of the 15 patients treated, all are considered evaluable. As expected, patients tolerated the tamoxifen well. One patient had weight gain with the compound. No patient had a sudden increase in discomfort or pain at the tumor site during initial therapy. Also, no patient had hypercalcemia during therapy. The patients were treated for a median of 12 weeks (range, six to 28).

One patient had a minor response in vaginal nodules with progression of tumor at other sites and three had stable disease for 3-6 months. Of these three patients, one felt there was improvement in her pelvic pain after starting the drug.

Of the 15 patients treated with tamoxifen, none achieved partial or complete response. Thus, the compound has minimal effectiveness in patients with re-

TABLE 1.—Patient characteristics

Characteristic	No. of patients (%)
Evaluable	15 (100)
Median age in yrs (range): 53 (29-69)	
Performance status (Zubrod)	
0	1 (7)
1	7 (47)
2	5 (33)
3	2 (13)
Prior progestational agents	1 (7)
Prior chemotherapy*	15 (100)
Metastatic sites	
Central/pelvic	11 (73)
Lung	3 (20)
Liver	1 (7)
Lymph node	6 (40)
Bone	1 (7)

*Median No. of regimens, 2.

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[1] Received Dec 20, 1985; accepted Feb 18, 1986.

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fractory glandular adenocarcinoma of the cervix who had received prior chemotherapy.

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CLINICAL TRIALS SUMMARIES

Phase II Evaluation of Mitolactol in Squamous Cell Carcinoma of the Head and Neck: A Southeastern Cancer Study Group Trial[1,2]

Michael S. McHale,[3] Enrique Velez-Garcia,[4] Owens Nelson,[5] Stephen D. Williams,[3] William Maddox,[6] and Robert Birch[6,*]

Mitolactol (DBD), a halogenated sugar alcohol with alkylating properties, has been tested in various hematologic and solid malignancies (1-4). Responses were noted in squamous cell carcinoma of the head and neck region in early studies (1). For this reason, we undertook a phase II study of DBD in this disease.

Eligible patients had histologically confirmed squamous cell carcinoma of the head and neck unsuitable for local treatment, measurable disease, Karnofsky performance status ≥ 50%, normal hepatic and renal functions, and adequate bone marrow reserve. DBD was given at a dose of 180 mg/m^2 orally at bedtime on Days 1-10 of each 28-day course. Subsequent courses were delayed if the granulocyte count was < 1500/mm^3 or the platelet count was $< 100{,}000$/mm^3. The dose was to be escalated to 190 mg/m^2 for subsequent courses if there was no significant toxicity. Blood cell counts were obtained every 2 weeks during the first course, then every 4 weeks for subsequent courses. Patients were evaluable for response after two courses of therapy. Partial response was defined as a $> 50\%$ reduction in the sum of the products of the largest perpendicular diameters of measurable disease.

Twenty-five patients were entered in this study (table 1). One patient refused therapy, no data were available on one patient, and one other patient died of pneumonia after one cycle of therapy, leaving 22 evaluable patients. Of these patients, one was evaluable for toxicity only.

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Entered	25
Unevaluable	1
Inadequate data	2
Evaluable	22
Median age in yrs (range): 58 (41-78)	
Sex	
Male	16
Female	6
Median Karnofsky performance status (range): 70% (50%-100%)	
Prior therapy	
Chemotherapy	1
Irradiation	2
Chemotherapy and irradiation	7
Chemotherapy, irradiation, and surgery	9
Irradiation and surgery	2
None	1
Extent of disease	
Locoregional	12
Below clavicle	10

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Five of these 22 patients had not received prior chemotherapy. There were no responses (95% confidence limits, 0%–18%). The mean number of cycles given was 1.7 (range, one to four). One patient received four cycles without toxicity. Toxicity was mainly myelosuppression. Seven patients had grade 3 (500–999 cells/mm^3) or 4 (< 500 cells/mm^3) granulocytopenia and five had grade 3 (25,000–49,999 cells/mm^3) or 4 (< 25,000 cells/mm^3) thrombocytopenia. All grade 3 and 4 toxicity occurred in patients who were previously treated with chemotherapy. Other toxic effects consisted of nausea, vomiting, and weight loss and were mild or absent. There were no drug-related deaths.

It is conceivable that higher doses of DBD in previously untreated patients would have induced responses. However, DBD at this dose and schedule in this patient population is without activity.

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CLINICAL TRIALS SUMMARIES

Combination Chemotherapy With Dacarbazine and Vindesine in the Treatment of Metastatic Malignant Melanoma[1,2]

Daniel A. Vorobiof,* Ricardo Sarli, and Geoffrey Falkson[3]

There are only three cytostatic agents for which consistent response has been claimed in advanced malignant melanoma, namely dacarbazine (DTIC), semustine, and vindesine (1-4). Our study was undertaken to investigate the value of DTIC and vindesine given concomitantly. Forty-six white patients with metastatic malignant melanoma who gave informed consent were entered on study. All had histologic confirmation of disease, an Eastern Cooperative Oncology Group (ECOG) performance status (PS) ≤ 3 (5), an adequate hemogram, and adequate liver and renal functions. No patients with brain metastases only were entered on study. The dose of DTIC was initially 400 mg/m^2 iv on Days 1-6 every 28 days for two cycles and thereafter 300 mg/m^2 iv on Days 1-5 every 28 days. Vindesine, 5 mg/m^2 iv on Days 1 and 6, was administered during the first two cycles and subsequently on Days 1 and 5. Hydrocortisone-sodium succinate, 300 mg/m^2, was given concomitantly iv. ECOG criteria for response were used (5). Patient characteristics, results, and toxic effects are presented in table 1.

The median duration of response was 20 weeks. The two complete responders had soft tissue metastases. One of these patients continues in complete response for > 120 weeks. In this study, the combination of DTIC and vindesine gave a response rate of 17% (95% confidence limits, 6%-28%). Patients with soft tissue metastases and/or only one site of metastasis and/or a PS of 0-1 at the time of entry on study had a better response rate and longer median duration of survival. Patients with prior chemotherapy had a lower response rate and a shorter survival time. Among previously untreated patients with a PS of 0-1, the response rate was 27% (six of 22 patients, 95% confidence limits, 9%-46%). The combination of DTIC and vindesine as used in this study has not given a response rate or survival time different from that expected with single agents.

TABLE 1.—Patient characteristics and treatment results

Characteristic	No. of patients
Total	46
Male	32
Female	14
Age (yrs) at entry	
Median: 46.5	
Range: 20-67	
Previous treatment	
Radiotherapy	1
Chemotherapy	11
Initial ECOG performance status*	
0	11
1	13
2	16
3	6
Extent of disease (No. of sites)	
1	11
2	21
≥ 3	14
No. of dominant disease sites	
Skin and soft tissue	48
Visceral	30
Central nervous system	14
Bone	13
Response (ECOG criteria)*	
Complete and partial	8
No change	19
Disease progression	17
Unevaluable	2
Toxicity†	
Anemia	16
Leukopenia	20
Thrombocytopenia	14
Nausea and vomiting	37
Alopecia	4
Neurologic toxicity (ECOG grade)*	
1 (mild paresthesias)	7
2 (severe paresthesias)	9

**See* ref 5.

†Toxicity data available for 40 patients.

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CLINICAL TRIALS SUMMARIES

Weekly Mitoxantrone in the Treatment of Advanced Pancreatic Carcinoma: A Southeastern Cancer Study Group Trial[1,2]

Philip A. DeSimone,[3,*] Richard Gams,[4] and Albert Bartolucci[5]

Mitoxantrone belongs to a new class of agents known as the anthraquinones, with a broad spectrum of activity in animal models (1). Below are the results of a phase II study conducted by the Southeastern Cancer Study Group (SECSG) in the treatment of advanced adenocarcinoma of the pancreas.

PATIENTS AND METHODS

Patients with histologically confirmed metastatic carcinoma of the pancreas no longer treatable with surgery and radiation therapy were eligible and were stratified into prior chemotherapy and no prior chemotherapy groups. All patients had the following: measurable disease; life expectancy of at least 6 weeks prior to starting chemotherapy; Karnofsky performance status $> 60\%$; caloric intake > 1200 kcals; adequate bone marrow reserve; absolute granulocyte count $> 2500/\text{mm}^3$; platelet count $> 125{,}000/\text{mm}^3$; bilirubin > 1.2 mg 100/ml; and written informed consent. Patients who had received prior chemotherapy must not have received any agents within 4 weeks (7 weeks for a nitrosourea). All patients were screened for co-existent heart disease. Patients who had received > 400 mg/m^2 of doxorubicin were eligible if they had normal radionuclide ejection fraction.

All patients received 5 mg/m^2 of mitoxantrone mixed in 250 ml of 5% dextrose in water over a 30-minute infusion. Six weekly injections were required for evaluation of response and toxicity. Patients with rapid progression after two weekly infusions were removed from the study. All toxic effects were graded according to SECSG toxicity criteria (2).

RESULTS

Thirty-five patients were entered in the study; 30 were evaluable for toxicity and response. Five patients were found to be inevaluable. The causes of inevaluability were: no confirmation of histology (one patient); only one injection of chemotherapy received (two); and no measurable disease (two). Thirteen of the evaluable patients gave a history of prior chemotherapy, while 17 had received no prior treatment. The diagnosis of pancreatic primary carcinoma was verified by surgical inspection and/or biopsy in all 30 evaluable patients. The treatment groups appeared to have differing sites of metastases (table 1). The group with prior chemotherapy had a predominance of liver disease, while the group with no prior chemotherapy had a predominance of nodal disease (mesenteric, aortic, and celiac). None of the 30 evaluable patients used computerized tomographic scan of the pancreas as the only measure of disease.

The schedule of mitoxantrone in this study produced varying degrees of hematologic toxic effects. An unexpected and unexplained finding was the number of severe reversible granulocytopenic episodes (absolute granulocyte count $> 1000/\text{mm}^3$), experienced in the no prior treatment group. No other hematologic toxic effects were noted. No patient in either group experienced infectious complications. There were no responses to therapy.

DISCUSSION

Reversible granulocytopenia was the main hematologic toxic effect, a finding consistent with that reported

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[2] This study was conducted by the Southeastern Cancer Study Group and was supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. The following institutions participated in the study: The University of Alabama at Birmingham (CA-03013, CA-19657, and CA-24456); Duke University, Durham, NC (CA-03177); Durham Veterans Administration Hospital, NC (CA-05634); The University of Miami, FL (CA-05641); Temple University, Philadelphia, PA (CA-07961); Washington University, St. Louis, MO (CA-03376); The University of Puerto Rico, San Juan (CA-12223); The University of Tennessee, Knoxville (CA-13237); Georgetown University, Washington, DC (CA-27587); City of Hope, Duarte, CA (CA-30247); Brooklyn Veterans Administration Hospital, NY; Case Western Reserve University, Cleveland, OH (CA-15584); The University of Louisville, KY (CA-16389); The University of Tennessee, Memphis (CA-17027); The University of Cincinnati, OH (CA-28138); The University of Florida, Gainesville (CA-28143); The University of South Florida, Tampa; St. Louis University, MO (CA-28169); The University of Kentucky, Lexington (CA-20255); Vanderbilt University, Nashville, TN (CA-23909); and The University of Medicine and Dentistry of New Jersey, Rutgers Medical School, Piscataway.

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TABLE 1.—Patient characteristics*

Characteristic	No prior chemotherapy	Prior chemotherapy
Evaluable	17	13
Mean age in yrs (range)	64.5 (47-77)	54.5 (27-75)
Sex		
Male	9	7
Female	8	6
Response	0	0
Site of measurable disease		
Liver	3	9
Lung	1	1
Lymph node	8	1
Miscellaneous	6	2
Toxicity		
Hematologic		
Absolute granulocyte count ($\times 10^3$ cells/mm^3)		
1.5–< 1.9	0	3
1.0–< 1.5	1	3
0.5–< 1.0	8	1
< 0.5	0	1
Gastrointestinal		
Nausea and vomiting (mild to moderate)	4	8
Diarrhea	3	0
Stomatitis	2	0

*Unless otherwise specified, values = No. of patients.

in the literature (3). The finding of severe granulocytopenia in patients with no history of prior chemotherapy is contrary to the findings of a recently completed study of the SECSG investigating weekly mitoxantrone in advanced colorectal and stomach carcinomas (2,4). The results of this study show that mitoxantrone is an ineffective agent in the treatment of advanced carcinoma of the pancreas.

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CLINICAL TRIALS SUMMARIES

Limited Activity of Carboplatin in Malignant Lymphoma[1,2]

Linda Danieu and Raymond P. Warrell, Jr[3,4,*]

Carboplatin is a new metal-based complex which has recently entered clinical trials. Preliminary studies have shown that this compound causes substantially less renal and gastrointestinal toxicity than cisplatin (1,2). Cisplatin has modest antitumor activity in lymphoma when used as a single agent (3-5). Therefore, we initiated a phase II study of carboplatin to evaluate the therapeutic utility of this drug in patients with advanced malignant lymphoma.

METHODS

Patient Eligibility

Patients were eligible if they had relapsed from or failed to respond to conventional treatment. Pretreatment requirements included: histologic confirmation of disease; Karnofsky performance status ≥ 50; bidimensionally measurable parameters of disease; no chemotherapy or irradiation within 3 weeks preceding treatment; wbc count $\geq 3500/\text{mm}^3$; platelet count $\geq 120{,}000/\text{mm}^3$; serum creatinine and total bilirubin concentration ≤ 1.5 mg/dl; and creatinine clearance ≥ 60 ml/minute.

Treatment Plan

Carboplatin was administered every 3 weeks at a starting dose of 350 mg/m^2. Subsequent doses were escalated by 50-mg/m^2 increments if hematologic toxicity was not observed (wbc count $\leq 3000/\text{mm}^3$ or platelet count $\leq 70{,}000/\text{mm}^3$). The dose was decreased by 50 mg/m^2 for nadir wbc counts $< 2000/\text{mm}^3$ or platelet counts $< 50{,}000/\text{mm}^3$. Treatment was withheld for 1 week if the platelet count was $< 75{,}000/\text{mm}^3$. One dose of carboplatin which produced hematologic toxicity was considered an adequate trial if no therapeutic response was observed. Standard response criteria were employed.

RESULTS

Twenty-three patients were treated. Patient characteristics are shown in table 1. All patients had received prior chemotherapy. Eighteen patients (75%) had also received radiotherapy. Three patients developed obviously progressive disease after receiving full doses of carboplatin without having achieved hematologic toxicity. Two of the remaining 20 patients (both of whom had diffuse large cell lymphoma) achieved partial response for 2 and 4 months, respectively.

Toxic Reactions

Thrombocytopenia was dose-limiting. Three patients required dose reduction due to this effect; subsequent doses of carboplatin were escalated in five patients. Treatment delay ($\geq$ 4 weeks) because of thrombocytopenia occurred in seven of 14 patients who received multiple doses. Leukopenia was less frequent, although two patients required hospitalization for fever related to neutropenia. Only two patients experienced nausea with vomiting. There was no significant renal toxicity. The median increase in serum creatinine concentration from baseline to peak values on study was 0.2 mg/dl. The baseline serum creatinine concentration was the highest value in the study for nine patients (38%). One patient developed a drug-related skin rash. No ototoxicity was observed.

DISCUSSION

We observed only two partial responses among 20 fully evaluable patients with Hodgkin's disease or non-Hodgkin's lymphoma. The observed percent response (10%; 95% confidence limits, 0%-23%) excludes three additional patients who developed progressive disease after receiving full doses of carboplatin without achieving criteria for hematologic toxicity. The statistical analysis is misleading to the extent that homogeneity of

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[4] We thank members of the Chemotherapy Research Nursing Staff for monitoring the drug infusions and Ms. Catherine Cassidy for database management.

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TABLE 1.—Characteristics and hematologic toxicity of patients with advanced lymphoma treated with carboplatin ($n = 24$)*

Characteristic		
Median age in yrs (range)	51	(18–72)
Median Karnofsky performance status	80	(50–90)
Median No. of prior chemotherapeutic regimens	2	(1–6)
Median No. of prior chemotherapeutic drugs	7	(3–12)†
Median No. of carboplatin doses	2	(1–4)
Median wbc count nadir ($\times 10^3$ cells/mm^3)	3.1	(0.5–12.1)
Median platelet count nadir ($\times 10^3$ cells/mm^3)	64	(6–378)
Histologic subtypes		
Diffuse large cell lymphoma	8	
Diffuse poorly differentiated lymphocytic lymphoma	1	
Diffuse mixed cell lymphoma	1	
Nodular poorly differentiated lymphocytic lymphoma	6	
Nodular mixed cell lymphoma	1	
Hodgkin's disease	6	

*Unless otherwise specified, values = No. of patients. Nos. in parentheses = range of values.

†No patient had previously received a platinum-containing compound.

the patient population is assumed. To circumvent the problem of response analysis in "negative" phase II trials of patients who have received extensive prior therapy, we followed patients who received carboplatin to determine their response to subsequent treatment. In 23 patients, there were one complete response and seven partial responses (35% response rate) to subsequent chemotherapy and/or irradiation. These data indicate that patients in this study were not refractory to potentially effective treatment. We conclude that the low observed percent response and the limiting toxic reaction (thrombocytopenia) suggest that carboplatin administered every 3 weeks offers no increase in therapeutic index relative to cisplatin for patients with advanced lymphoma.

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LETTERS

High Incidence of Veno-occlusive Disease Related to Indicine *N*-Oxide in the Treatment of Refractory Adult Acute Leukemia

Indicine *N*-oxide (INO) is a pyrrolizidine alkyloid that in early clinical studies had shown promise in the treatment of acute leukemia refractory to established therapy (1). To better define the efficacy of this agent in treating refractory acute leukemia in adults, a phase II trial was initiated by the Southeastern Cancer Study Group in 1982. After five patients had been entered in the protocol, the study was suspended in 1983 when four of the five treated developed liver toxicity suspected to be drug-related. An additional patient, treated with the same regimen under a special exception protocol of the National Cancer Institute prior to activation of the Group study, appeared to have similar liver toxicity and is included in this report. Four of the five patients with suspected drug-related liver toxicity have had an autopsy and review of the liver pathology is consistent with veno-occlusive disease (VOD) in the four patients. While VOD is a recognized complication of INO (2), its frequency and severity in this population were more than expected and appear to limit the clinical utility of this agent. We wish to briefly review the characteristics of the patients treated, the treatment regimen, and the clinical and pathological evidence of VOD.

The six patients ranged in age from 22 to 69 years. Three patients had acute myeloblastic leukemia (AML), two had lymphoblastic leukemia (ALL), and one had biphenotypic leukemia (AML-ALL) (table 1). All patients had received extensive prior chemotherapy, and the three patients with AML had previously received sequentially administered 5-azacitidine and amsacrine. No patient had significant liver dysfunction at the initiation of treatment (table 1). A course of therapy consisted of INO (3000 mg/m^2) infused over 5 minutes daily for 5 days. If the marrow remained hypercellular with leukemia after 15 days from initiation of therapy, a second course of INO could be given.

Marrow aplasia and clearing of peripheral blood blasts occurred in three patients (two with AML; one with ALL). All patients developed severe pancytopenia.

Changes in liver chemistries first appeared between 21 and 36 days after entry in the protocol, and in three patients were accompanied by right upper quadrant pain and the development of ascites. Autopsy material was available for five of the patients and was reviewed by one of us. Histopathologic examination of the livers showed similar morphology in four patients and consisted of edema, congestion, and hepatocellular necrosis, strikingly limited to central venous areas (3). Occlusion of the hepatic vein branches was not identified. The fifth patient (No. 6) showed fibrosis of the central veins and intimal proliferation of several sublobular veins. There was no venous occlusion or centrilobular necrosis.

The frequency and severity of VOD seen in these patients were not expected based on data available when this study was initiated (1,4). Dose-limiting hepatotoxicity was not seen in earlier phase I-II studies involving patients with solid tumors, even with the identical dosing schedule employed in this study (4). While there was a suggestion that patients with previously refractory leukemia may be more susceptible to hepatotoxicity, this had been associated with a slightly higher dosage (3.37 and 3.75 g/m^2 daily × 5) (1,5). Our findings of unacceptable hepatotoxicity in this patient population at the 3-g/m^2 daily × 5 dosage are consistent with subsequent observations in a larger study involving heavily pretreated pediatric patients (J. Miser, personal communication). INO appears to be an attractive drug because it represents a new class of compounds and has

TABLE 1.—Patient characteristics and INO-related hepatotoxicity[*]

					Liver chemistry changes					
Patient No.	Diagnosis	Age (yrs)	Sex	No. of INO courses	Bilirubin (mg/dl)	SGOT (U/L)	Alkaline phosphatase (U/L)	Ascites	Survival (days)	VOD on autopsy
1	AML	69	M	1	18.0 (0.7)	78 (17)	150 (86)	+	26	+
2	AML	45	M	2	33.4 (0.6)	244 (101)	208 (95)	+	49	ND[†]
3	AML	33	F	2	5.6 (0.1)	330 (14)	4360 (119)	+	48	+
4	ALL-AML	23	F	2	8.1 (0.4)	122 (17)	120 (65)	+	39	+
5	ALL	30	M	1 + 3/5	1.1 (1.6)	640 (27)	165 (97)	+	21	+
6	ALL	32	F	4	2.8 (0.2)	92 (22)	92 (70)	−	96	−

[*] Nos. in parentheses = pretreatment liver chemistries.

[†] ND = not done.

demonstrated antitumor activity which includes clinical regression in ALL. However, further studies using the dose and schedule of INO described in patients with refractory leukemia do not appear justified and reduced dosage would be expected to be less therapeutic. Whether other pyrrolizidine alkyloids or synthetic analogs can separate the antineoplastic from the hepatotoxic effects of this class of compounds is a matter for further study.

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Effect of Diphenhydramine on the Antiemetic Action of Metoclopramide Against Cisplatin-Induced Emesis in Dogs

Recent studies (1) suggested that the antiemetic effect of metoclopramide (MCP) against cisplatin-induced emesis was due in part to its gastrokinetic properties via a cholinergic mechanism and not due to a dopamine-blocking action. Clinically, diphenhydramine (DPH), an antihistamine with anticholinergic properties, is often given to control or prevent the occurrence of extrapyramidal symptoms sometimes observed with MCP in patients undergoing cancer chemotherapy (2-5). This study was undertaken to determine whether DPH, due to its anticholinergic properties, would alter the antiemetic effect of MCP against cisplatin-induced emesis in dogs.

In two separate studies, groups of four dogs received iv MCP either alone or combined with DPH 60 minutes following the iv injection of 3.0 mg/kg of cisplatin. All animals were observed for emesis for 5 hours following the administration of the drugs.

The results in table 1 indicate that DPH at 1.0 mg/kg iv, a dose equivalent to that used in patients undergoing

TABLE 1.—Effects of DPH on the antiemetic action of MCP against cisplatin-induced emesis in dogs

Treatment	Dose* (mg/kg iv)	Emetic episodes (mean ± SD)	% change from control
Experiment 1			
Control (saline)	0.1 ml/kg	9.3 ± 6.2	
DPH HCl	1.0	12.0 ± 5.9	+30
MCP HCl	3.16	2.3 ± 2.6†	−76
	5.7	1.0 ± 1.4†	−89
	10.0	2.0 ± 1.4†	−78
MCP HCl + DPH HCl (1.0 mg/kg iv)	3.16	2.8 ± 2.1†	−70
	5.7	1.3 ± 1.0†	−87
	10.0	1.3 ± 1.9†	−87
Experiment 2			
Control (saline)	0.1 ml/kg	17.3 ± 8.9	
DPH HCl	3.16	10.8 ± 3.8	−38
MCP HCl	3.16	2.5 ± 3.1†	−86
	5.70	1.5 ± 1.3†	−91
	10.0	4.3 ± 7.2†	−75
MCP HCl + DPH HCl (3.16 mg/kg iv)	3.16	4.0 ± 2.4†	−77
	5.7	1.0 ± 1.4†	−94
	10.0	3.5 ± 6.4†	−80

*Expressed as the base; 4 animals/group.
†Significantly different from control, $P < 0.05$; Dunnett's t test.

cancer chemotherapy, and at 3.16 mg/kg iv did not significantly alter the antiemetic effects of MCP against cisplatin-induced emesis. Further, as seen clinically, DPH reduced the central nervous system side effects of MCP in the dogs, ie, pawing, head shaking, and cage biting. Results from our laboratories indicate that 3 mg/kg iv of DPH was sufficient to inhibit the gastrokinetic effects of MCP. This suggests that the gastrokinetic action of MCP does not markedly contribute to its antiemetic effect. We conclude that antiemetic properties of MCP against cisplatin-induced emesis in dogs are not altered by DPH at doses as high as three times the clinical dose that is used in patients undergoing cancer chemotherapy. We suggest that using DPH in patients to control the extrapyramidal symptoms seen with MCP will not affect its antiemetic properties.

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December 23, 1985

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Treatment of Refractory Acute Lymphoblastic Leukemia With Teniposide and Cytarabine

Chemotherapy with vincristine, prednisone, and L-asparaginase (with or without an anthracycline) for acute lymphoblastic leukemia achieves remission in > 90% of patients. However, treatment for the few patients who do not initially achieve a remission and for the children who have had one or more bone marrow relapses is not as successful. This is most likely due to the development of resistance to the most effective drugs available. Teniposide (VM-26) and cytarabine (Ara-C) are active drugs against acute lymphocytic leukemia (1,2). Rivera et al (3) reported that ten of 33 children who had failed primary induction or had multiple relapses achieved complete remission when given VM-26 and Ara-C. There has been no confirmation of their data. In this report we confirm Rivera's observations. However, our initial attempts at using VM-26 plus Ara-C for maintenance treatment produced only short remissions.

PATIENTS AND METHODS

Patients

Patients had acute lymphoblastic leukemia (two with T-cell and 27 with common or non-T, non-B cell) and were between 2 and 21 years of age. Twenty-eight patients had relapsed one to seven times (median, three); 26 of these 28 had failed initially during or < 6 months after stopping initial multidrug therapy at this (22 patients) (4,5) or other centers. One patient had failed initial therapy with prednisone, vincristine plus L-asparaginase, and then the combination of doxorubicin plus Ara-C. The patients had a life expectancy of at least 4 weeks, had recovered from toxicity of prior treatment, and had adequate liver and renal functions (bilirubin < 2 mg/100 ml; creatinine < 1.5 mg/100 ml). Patients with CNS leukemia received concomitant intrathecal methotrexate for treatment of their CNS disease. Complete remission was defined as an absolute granulocyte count $> 1500/\text{mm}^3$, a hemoglobin > 10 g/100 ml, a platelet count $> 100{,}000/\text{mm}^3$, and an M_1 marrow. All patients signed informed consent.

Treatment

VM-26 (165 mg/m^2) was diluted to 1 mg/ml in a solution of 5% dextrose in water in 0.25 normal saline and administered as a 30–40-minute iv infusion. Ara-C (300 mg/m^2) diluted to a concentration of 50–100 mg/ml in the supplied diluent was then given as an iv bolus infusion. The two drugs were given twice weekly (Monday–Thursday or Tuesday–Friday). The initial protocol specified eight doses of drug over 4 weeks. However, because of profound hypoplasia after four doses, the protocol was modified. A bone marrow aspirate was obtained prior to the fifth dose. If the bone marrow was aplastic with no evidence of leukemia, patients were then allowed to recover marrow function before resumption of treatment. If there was residual leukemia an additional drug dose was given and the marrow analysis repeated before the next scheduled dose of VM-26 and Ara-C.

RESULTS

Two patients had immediate reactions with the first dose of VM-26 consisting of mild wheezing, chest pains, urticaria, and hypotension. They were therefore removed from the study. The other 27 patients received a total of 184 doses of VM-26 and Ara-C. The mean number of doses was five (range, two to eight) during initial induction attempts. There were nine complete remissions (including the one patient who had failed initial induction). An additional three patients with hypoplastic but recovering bone marrows received bone marrow transplants before full documentation of M_1 status was established. One other patient with a recovering but hypoplastic marrow died of fungal sepsis. Six patients had minor responses (decreased or complete clearing of peripheral blasts). The remaining eight patients had no response; five had six doses and three had five doses before there was obvious disease progression. The mean time to M_1 marrow following the last dose of VM-26 and Ara-C was 3 weeks. The median number of doses given to the nine patients who achieved complete response was five (range, three to seven doses). The median number of prior relapses in the group achieving complete response was three (range, two to seven relapses). Patients with no response had a median of two prior relapses (range, one to four).

Children in complete remission who were not candidates for bone marrow transplant received maintenance therapy with two to four doses of VM-26 and Ara-C (twice weekly schedule) every 2–3 weeks, depending on peripheral blood cell counts. All patients relapsed within 3 months. One of the three children who were transplanted with hypoplastic but recovering marrows is alive and disease-free 5 years later. The other two children relapsed and died.

Toxic effects were related to profound myelosuppression. All patients who achieved complete remission were hospitalized during the induction period for treatment of documented or suspected infection. They were also transfusion-dependent for platelets and rbcs. Diarrhea was moderate to severe in all patients. Hospitalized patients required support with total parenteral nutrition. Except for the two patients in whom anaphylaxis was seen with the first dose, hypotension, nausea, vomiting, and other untoward reactions were minimal.

DISCUSSION

The complete remission rate observed here (nine responses among 27 patients) confirms the initial observations of Rivera et al (3) in patients with multiple-relapsed acute lymphoblastic leukemia. VM-26 plus Ara-C is an effective, albeit toxic, regimen for remission induction in children with acute lymphoblastic leukemia.

Despite continued use of VM-26 plus Ara-C in maintenance therapy, remission durations were not very long. We are currently investigating the use of VM-26 and Ara-C in combination with another known effective drug pair (methotrexate and L-asparaginase) during the continuation phase of therapy. The efficacy of VM-26 and Ara-C for maintenance therapy for acute lymphoblastic leukemia requires further controlled studies.

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Destruction of Both Extensive Local Recurrent and Primary Breast Cancer With Topical Aqueous 5-FU: A Clinical Observation

Over the last 6 years we have been treating locally recurrent and primary ulcerating breast carcinoma that is not resectable and either radiation-resistant or recurring in an area which has been given maximum amounts of radiation with an aqueous solution of 5-FU applied topically. This has resulted in a rapid selective destruction of tumor tissue, which occurs to a greater depth than would be anticipated, considering that in normal skin the depth of penetration of a topically applied drug is usually limited.

PATIENTS AND METHODS

Seven patients with histologically proven breast cancer were treated. The tumor panus varied from 20% of the width of the anterior chest wall to 65% of the chest wall. Tumor thickness varied from 0.3 to 2.0 cm. All patients had received prior radiation therapy to what would be considered a maximum tolerable dose. The tumor either was resistant to radiation or occurred in a previously irradiated area. Five of the patients had undergone mastectomy, while one had undergone automastectomy secondary to extensive tumor growth over many years. All patients had received hormonal therapy if appropriate and chemotherapy. Aqueous 5-FU was prepared by diluting ampules of the substance with distilled water. The concentration was varied between 0.5% and 5.0%. A thick dressing (1 cm), large enough to cover the entire lesion, was thoroughly impregnated with the aqueous 5-FU solution, placed on the involved area, and covered with polyethylene film (Saran Wrap) to prevent evaporation. This was allowed to remain in place for 8–12 hours. At about 4–6 hours more solution was added. The patients with a tumor thickness < 0.3 cm were treated with a 0.5% 5-FU solution. One percent solution was used if the tumor was < 0.5 cm and 3.0% solution was used if the tumor was between 0.5 and 2 cm in thickness. The patient with massive chest wall involvement was treated with the 5.0% solution. Ea[illegible] tumor-bearing area was given one course of therapy. The superficial tumors were treated for 10 days. The patients with extensive tumor were treated for 3 weeks.

RESULTS

Six of the seven patients had complete necrosis of the treated tumor tissue. Frequently, discrete lines of demarcation could be seen between the tumor and the normal tissue. Destruction of the tumor-bearing area was visually evident within 48 hours; this was characterized by the development of blanching followed by an opalescence and then necrosis when the tumor panus was < 0.3 cm thick. In thicker tumors opalescence was not clearly evident. Two patients had tumor masses as great as 2.0 cm in thickness, which nevertheless responded rapidly to the aqueous 5-FU as manifested by tumor necrosis. After stopping 5-FU, four of the treated patients had complete epitheliazation within 10 days following tumor destruction. The fifth patient with extensive disease required 6 weeks for epitheliazation. Four of the six responders had metastasis in other areas including the lungs and died within 4 weeks of treatment. The patient with the total breast necrosis was lost to follow-up after the disappearance of tumor and complete re-epitheliazation. One patient who initially responded and then developed recurrence of tumor is being re-treated with a 5-FU-containing regimen.

Leukopenia and stomatitis were not seen in any of the patients. The only complication noted was persistent oozing of blood from the treated site in one patient who had thrombocytopenia.

Our use of various concentrations of 5-FU was predicated on the concept that higher concentrations would be needed to effectively permeate a large tumor mass and diffuse into the tumor cells deep within its structure. The commercially available topical 5-FU products did not cause significant tumor destruction in any of our patients.

DISCUSSION

We have known for many years that the surface bonding between tumor cells is not as strong as that between cells in normal tissue. Tumor tissue has increased amounts of intercellular water, fluid, and protein. This results in a more effective perfusion of the tumor mass and the exposure of a greater cell surface area to the passage of substances into the cell. The evidence for this is that tumor nodules are known to survive and grow without the presence of a blood supply because of the more efficient perfusion of the tumor by the surrounding fluids.

Based on these observations it appears that breast tumor tissue is easily penetrated by aqueous 5-FU with a rapid onset of action, resulting in the selective destruction of cancer cells.

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Rectal Administration of Medroxyprogesterone Acetate in Cancer Patients: A Pharmacokinetic Study

Medroxyprogesterone acetate (MAP) is a synthetic progestational agent widely used in metastatic breast cancer therapy (500–5000 mg/day) (1). Both im and oral administrations are currently employed, but these two administration routes are not equipotent (2).

MAP is nearly insoluble in water and in human plasma (1–2 mg/ml) and this strongly influences its rate of absorption, which is primarily dependent on the available surface area of exchange between the body fluids and the administered drug, and, after oral treatment, on the period of time it is maintained in the gastrointestinal tract. Only a very limited amount of drug

TABLE 1.—MAP plasma levels observed in patients after single 500-mg rectal administration or after single 500-mg oral treatment*

	Rectal administration (ng/ml)				
Time (hrs)	Patient 1	Patient 2	Patient 3	Patient 4	Oral treatment (mean ng/ml)†
0	0.0	0.0	0.0	0.0	0.0
1	0.7	0.8	0.4	0.3	6.7
2	1.6	1.0	0.3	0.5	9.9
4	2.0	1.2	0.4	0.8	9.4
8	3.3	1.1	0.6	0.4	3.0
24	2.8	1.0	0.0	0.0	2.0
48	—	0.0	0.0	0.0	1.5
72	0.0	0.0	0.0	0.0	1.2

*Because of these unfavorable results, we do not deem the rectal administration of MAP a possible alternative to the currently established treatments.

†$N = 10$.

is absorbed after oral administration (0.2%–17.4% of the administered dose) and the release of MAP from the injection site is quite slow (7.0–38.5 mg/day for a 500-mg im injection) (3).

Sometimes the administration of MAP is impossible either by oral or by im treatment due to persistent vomiting in gastrointestinal tumors and local intolerance, respectively. For this reason, we have studied MAP plasma levels after a single rectal administration of 500 mg in cocoa butter suppositories. The subjects of this study were four hospitalized patients with advanced cancer. All patients were assessed as having normal liver and renal functions by standard clinical tests. No other treatment was carried on during this study to avoid any pharmacological interference. Venous blood samples for MAP assay were drawn with heparinized syringes. Plasma (1 ml) was extracted and analyzed following a specific automated gas-chromatographic method previously described (4).

Table 1 shows the plasma levels observed in this study compared with mean values obtained after a single oral treatment of the same dose (ten cancer patients) (4). The results indicate a quite erratic and negligible absorption of the drug, as seen in all patients.

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ANNOUNCEMENTS

8th Congress of the European Society of Parenteral and Enteral Nutrition

Paris, France, September 14-17, 1986

The 8th Congress of the European Society of Parenteral and Enteral Nutrition will be held in Paris at the Palais des Congres, Porte Maillot, on September 14-17, 1986. The first day, Monday, September 15, will be devoted to an educational session for physicians, pharmacists, dieticians, and nurses. The scientific sessions, Tuesday and Wednesday, will consist of two honorary lectures, two symposia, and three workshops. The educational sessions will be presented in French, with simultaneous translation to English; the scientific sessions will take place in English, with translation to French.

The Sir David Cuthbertson Lecture will be given by Professor E. Jequier on the "Influence of Nutrient Administration on Energy Expenditure," and the Arvid Wretlind Lecture will be given by Professor D. B. S. Silk on "Towards Optimal Enteral Nutrition."

The programs of the symposia and workshops have been designed to respond to the following questions: control of nitrogen metabolism, the relationship between parenteral nutrition and respiratory function, the importance of preoperative nutrition, digestive adaptation to artificial nutrition, and the present status of home parenteral nutrition in Europe. Parallel sessions will allow for oral communications and poster presentations.

Deadline for submission of abstracts is *April 1, 1986.* Registration fees for the entire congress are as follows: Member (ESPEN, SFNEP, and ASPEN)—prior to June 15, 1986, 950 FF; after June 15, 1986, 1350 FF; Nonmember—prior to June 15, 1986, 1300 FF; after June 15, 1986, 1700 FF. For a detailed breakdown of congress fees other than those already listed, contact: Claude Ricour, *see* address below.

This meeting should meet the criteria for credit hours of the Physician's Recognition Award of the AMA.

For further information, contact: Claude Ricour, Scientific Secretariat, ESPEN 86 149, rue de Sevres, 75743 Paris Cedex 15, France.

International Congress on Tumoral Markers

Naples, Italy, September 18-19, 1986

The International Congress on Tumoral Markers will be held September 18-19, 1986, at the Castel Dell-Ovo, Naples, Italy.

For further information, contact: OOMER Secretariat, ICIG, Hôpital Paul-Brousse, 14-16 Ave Paul-Vaillant-Couturier, 94800 Villejuif, France (telephone: 46.77.00.00, ext 306).

Second Annual Symposium of the Oncologic Organization of Mediterranean Education and Research (OOMER)

Naples, Italy, September 20, 1986

Cancer epidemiology in Mediterranean countries will be the topic of the Second Annual Symposium of OOMER. Professor Georges Mathé is the organizer.

For further information, contact: OOMER Secretariat, ICIG, Hôpital Paul-Brousse, 14-16 Ave Paul-Vaillant-Couturier, 94800 Villejuif, France (telephone: 46.77.00.00, ext 306).

IVth Tissue Culture Association Decennial Review—Cell and Tissue Culture International Conference on Molecular Mechanisms in the Regulation of Cell Behavior

Hershey, Pennsylvania, September 22-26, 1986

The IVth Tissue Culture Association Decennial Review—Cell and Tissue Culture International Conference on Molecular Mechanisms in the Regulation of Cell Behavior is scheduled for September 22-26, 1986, at Hershey, Pennsylvania. The program features formal papers, open poster presentations, workshops, and commercial exhibitors. Preregistration ends on *July 22, 1986.*

For further information, contact: Executive Director, Tissue Culture Association, 19110 Montgomery Village Ave, Suite 300, Gaithersburg, MD 20879 (telephone: 301-869-2900).

Urologic Cancer

Boston, Massachusetts, September 22-24, 1986

This intensive, 3-day course will provide an overview of the clinical and pathophysiological basis for the diagnosis and treatment of patients with urologic cancer. This course will emphasize the multidisciplinary approach which constitutes the foundation of contemporary care for patients with urologic cancers. Principles derived from the disciplines of urologic surgery, medical oncology, and radiation therapy will be highlighted and incorporated into a comprehensive consideration of therapeutic practices. While teaching will be primarily provided through lectures, there will be an emphasis on panel discussion of both problem management cases and controversies in urologic cancer. Specifically, definitive lectures and panels highlighting controversies in care will be provided for prostate cancer; bladder cancer, with a special emphasis on newer therapeutic modalities; and testicular cancer. Intensive interaction with the faculty will be provided by question and answer periods, as well as informal lunches attended by both faculty and students. In addition, a comprehensive syllabus will be provided.

Guest faculty will include Drs. Malcolm A. Bagshaw, John P. Donohue, Lawrence H. Einhorn and Derek Raghavan. The Harvard/local faculty includes leaders in urologic cancer from the Dana-Farber Cancer Institute, Brigham and Women's Hospital, Massachusetts General Hospital, Lahey Clinic, University of Massachusetts Medical Center, and Rhode Island Hospital.

Tuition is $390, with a reduced fee of $275 for fellows and residents.

For further information, contact: Harvard Medical School, Department of Continuing Education, Boston, MA 02115 (telephone: 617-732-1525).

Bone and Cancer: International Congress in Hanasaari

Helsinki, Finland, October 9-11, 1986

For further information, contact: European Forum for Orthopaedic Science Congress Bureau, PO Box 4, SF-00661 Helsinki, Finland.

Ninth Annual Bristol-Myers Symposium on Cancer Research

Washington, DC, October 15-16, 1986

The Vincent T. Lombardi Cancer Research Center of Georgetown University School of Medicine is organizing the Ninth Annual Bristol-Myers Symposium on Cancer Research. This year's topic will be "Mechanisms of Drug Resistance in Neoplastic Cells." Sessions include: general considerations of drug resistance; enzymatic basis of drug resistance; multidrug resistance; and the circumvention of resistance.

The annual Bristol-Myers symposium is part of an $8.34 million program of unrestricted grants for cancer research the company has sponsored since 1977 at 17 institutions in the United States, Canada, Europe, and Japan.

For more information, contact: Ms. Lillian Kamal, Administrator, Vincent T. Lombardi Cancer Research Center, Georgetown University School of Medicine, 3800 Reservoir Rd, NW, Washington, DC 20007.

Fifth NCI-EORTC Symposium on New Drugs in Cancer Therapy

Amsterdam, The Netherlands, October 22–24, 1986

This symposium will be organized by the European Organization for Research on Treatment of Cancer (EORTC) and by the National Cancer Institute (NCI), National Institutes of Health. The following topics will be discussed: screening and pharmacology, formulation, phase I trials and clinical pharmacology, phase II trials of new cytostatic agents, new analogs, and biological response modifiers.

For further information, contact: Symposium Secretariat, EORTC-New Drug Development Office, Department of Oncology, Free University Hospital, De Boelelaan 1117, NL-1081 HV Amsterdam, The Netherlands [telephone: (20) 548-5193, 548-5192].

Cincinnati Cancer Conference V: Breast Cancer

Cincinnati, Ohio, October 31–November 1, 1986

The Cincinnati Cancer Conference V: Breast Cancer is being sponsored by the Bethesda Hospital, Inc, the American Cancer Society, and the University of Cincinnati College of Medicine. The objectives are as follows: upon completion of the CME activity, physicians will be able to demonstrate ability to describe the role of various treatment modalities in breast cancer; discuss methods of detection of breast cancer; and apply the results of clinical research to patients with breast cancer.

Featured faculty are as follows: Gianni Bonadonna, Stephen K. Carter, Caldwell B. Esselstyn, Jr, Gilbert H. Fletcher, Jay R. Harris, John A. Katzenellenbogen, Marc E. Lippman, David P. Rose, Philip Strax, and Douglass C. Tormey.

For further information, contact: Tom O'Connor, Course Manager, Bethesda Hospital, Inc, 619 Oak St, Cincinnati, OH 45206 (telephone: 513-569-6339).

Chemotherapy Foundation Symposium VII—Innovative Cancer Chemotherapy for Tomorrow

New York, New York, November 12–14, 1986

The Sheraton Centre Hotel in New York City will be the location for the Chemotherapy Foundation Symposium VII—Innovative Cancer Chemotherapy for Tomorrow, to be held November 12–14, 1986. Individual sessions will be devoted to postmenopausal breast cancer polychemotherapy, differentiation chemotherapy, chemoimmunotherapy, and new phase II drugs.

The 3-day symposium is being sponsored by the Division of Medical Oncology, Department of Medicine, Department of Neoplastic Diseases, and the Page and William Black Post-Graduate School of Medicine of the Mount Sinai School of Medicine (CUNY).

For further information, contact: Director, The Page and William Black Post-Graduate School of Medicine, One Gustave L. Levy Place, New York, NY 10029 (telephone: 212-650-6772).

AIDS and the Nervous System

San Francisco, California, December 11–12, 1986

This symposium, located in the St. Francis Hotel and sponsored by the University of California in San Francisco, is designed to present the most recent information on the care of AIDS patients with neurological disease including: overview and neuroepidemiology, clinical syndromes and diagnosis, primary and opportunistic viral infections, nonviral infectious disease, Kaposi's sarcoma and systemic lymphomas, patient care issues, and recent research developments. Ten hours of ACCME/AMA/CMA Category 1 credit may be earned by attending the symposium.

For further information, contact: Extended Programs in Medical Education, Rm 569-U, University of California, San Francisco, CA 94143 (telephone: 415-476-4251).

International Symposium on Breast Cancer

New Delhi, India, January 1-4, 1987

The Tata Memorial Center, Bombay, and Association of Surgeons of India are jointly sponsoring an International Symposium on Breast Cancer to be held from January 1-4, 1987, at the Taj Palace Hotel, New Delhi, India. The symposium will address the current state of the art in all aspects of breast cancer including basic research that is of interest to the clinician.

For further information, contact: Dr. I. Mittra, Organising Secretary, Ernest Borges Marg, Parel, Bombay 400012, India.

5th International Conference on the Adjuvant Therapy of Cancer

Tucson, Arizona, March 11-14, 1987

The 5th International Conference on the Adjuvant Therapy of Cancer will be held at the Tucson Convention Center, Wednesay, March 11, through Saturday, March 14, 1987. The Arizona Cancer Center is sponsoring the conference. Sydney E. Salmon will serve as conference chairman. The scientific advisory committee members are Vincent DeVita, Jr, Bernard Fisher, Emil Frei, III, and Stephen E. Jones.

The deadline for submission of abstracts (prepared in the format of AACR/ASCO) is *December 1, 1986.*

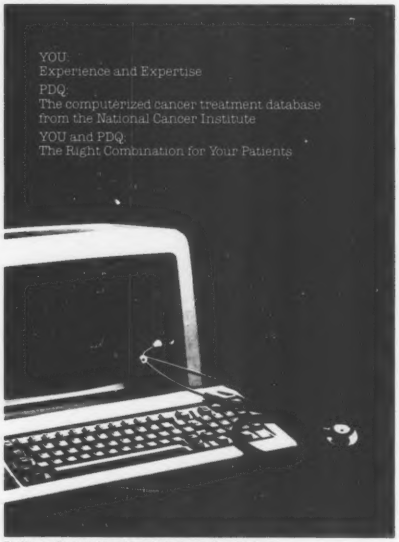
For further information, contact: Mary Humphrey, Conference Coordinator, Arizona Cancer Center, The University of Arizona College of Medicine, Tucson, AZ 85724 (telephone: 602-626-6044).

Third International Conference on Malignant Lymphoma: Current Status and Prospects

Lugano, Switzerland, June 10-13, 1987

The Palazzo dei Congressi is the location for this series of lectures and workshops dealing with the diagnosis and treatment of Hodgkin's and non-Hodgkin's lymphoma in adults and children. Most lectures will be given by invited speakers. The program will include free presentations, workshops, and a poster session. The deadline for the receipt of abstracts is *January 15, 1987.*

For further information, contact: F. Cavalli, MD, Division of Oncology, Ospedale San Giovanni, 6500 Bellinzona, Switzerland (telephone: 091-23-62-10).



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BRIEF REPORTS

891 Etoposide and High-Dose Cisplatin in Good-Risk Patients With Advanced Squamous Cell Carcinoma and Adenocarcinoma of the Lung

893 Cisplatin Plus Etoposide as Second-Line Treatment in Advanced Ovarian Carcinoma

897 Treatment of Advanced Gastric Carcinoma With Mitomycin and Doxorubicin

899 High-Dose Mitoxantrone in Metastatic Breast Cancer: A Phase I-II Trial

903 Plasma Pharmacokinetics of 5-FU Given by Continuous Infusion With Allopurinol

905 Lhermitte's Sign as a Complication of Cisplatin-Containing Chemotherapy for Testicular Cancer

909 Phase II Study of Cisplatin, 5-FU, and Allopurinol in Advanced Esophageal Cancer

CLINICAL TRIALS SUMMARIES

911 Phase II Evaluation of Oral Idarubicin (4-Demethoxydaunorubicin) in Patients With Disseminated Malignant Melanoma

913 Guanazole in the Treatment of Advanced Bronchogenic Carcinoma: A Pilot Study of the Southeastern Cancer Study Group

915 Treatment of Advanced Adenocarcinoma of the Kidney With ICRF-187: A Southeastern Cancer Study Group Trial

917 Phase II Eastern Cooperative Oncology Group Study of Spirogermanium in Previously Treated Lymphoma

919 Ifosfamide Plus *N*-Acetylcysteine in the Treatment of Small Cell and Non-Small Cell Carcinoma of the Lung: A Southeastern Cancer Study Group Trial

921 Phase II Evaluation of Mitoxantrone in Advanced Renal Cell Carcinoma: A Southeastern Cancer Study Group Trial

923 Tamoxifen in the Treatment of Refractory Adenocarcinoma of the Cervix

925 Phase II Evaluation of Mitolactol in Squamous Cell Carcinoma of the Head and Neck: A Southeastern Cancer Study Group Trial

927 Combination Chemotherapy With Dacarbazine and Vindesine in the Treatment of Metastatic Malignant Melanoma

929 Weekly Mitoxantrone in the Treatment of Advanced Pancreatic Carcinoma: A Southeastern Cancer Study Group Trial

931 Limited Activity of Carboplatin in Malignant Lymphoma

LETTERS

933 High Incidence of Veno-occlusive Disease Related to Indicine *N*-Oxide in the Treatment of Refractory Adult Acute Leukemia

934 Effect of Diphenhydramine on the Antiemetic Action of Metoclopramide Against Cisplatin-Induced Emesis in Dogs

935 Treatment of Refractory Acute Lymphoblastic Leukemia With Teniposide and Cytarabine

936 Destruction of Both Extensive Local Recurrent and Primary Breast Cancer With Topical Aqueous 5-FU: A Clinical Observation

937 Rectal Administration of Medroxyprogesterone Acetate in Cancer Patients: A Pharmacokinetic Study

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